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AN ANALYSIS OF CERTAIN PHOTIC REACTIONS, WITH REFERENCE TO THE WEBER-FECHNER LAW

I. The Reactions of the Blowfly Larva to Opposed Beams of Light

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I. STATEMENT OF PROBLEM

The problem dealt with in this paper is the analysis of a series of measurements made on the motor reactions of the blowfly larva to light.

The measurements were obtained by a method already used with satisfactory results (8), in which light was applied as two opposed beams the intensity of which could be easily controlled and precisely measured. The reactions produced by two beams of light of different intensities, acting simultaneously on opposite sides of the same animal, were measured in angular deflections from an original path of locomotion. Since no deflection was produced when the opposed lights were of equal intensity, and since a deflection appeared and increased progressively as the intensity difference between the lights was increased, the relative difference between the intensities of the opposed lights was regarded as the value of the stimulus. Some five thousand trails were measured and the results expressed graphically in the form of curves in which the amplitude of the reaction was plotted against the intensity of the stimulus.

The analysis of these curves was undertaken to ascertain, if possible, the factors that determine the relation between reaction amplitude and stimulus intensity. Because there has been considerable work done on the so-called Weber-Fechner relationship between *"sensation value"* and stimulus intensity, it seemed desirable to consider the measurements presented here with reference to the Weber-Fechner Law. Such a method of treatment offers the possibility of interesting comparisons between *reaction* and *sensation* curves, and at the same time gives a definite point of view from which to approach an analysis of the data.

II. INTRODUCTION

The relationship between the strength of a stimulus and the response evoked by it was first systematically studied by Weber about the middle of the last century (11, 12). The criterion by which he measured the effect of the stimulus was "the least detectable change in sensation," a method necessarily limited to human physiology. His experiments showed that the increase in stimulus necessary to cause the least detectable change in sensation is, within a certain medium range, a definite fractional increment of the acting stimulus. Fechner (2) attempted to give this law a more quantitative and extensive application by assuming that just perceptible differences in sensation represent actually equal amounts of sensation. Accepting this assumption, we may express the relation between stimulus and sensation, as determined by Weber's experiments, as follows: in order to increase the sensation by equal amounts, that is, in arithmetical progression, the stimulus must be increased according to a certain factor, that is, in geometrical progression. The sensation may therefore be regarded as a geometrical function of the stimulus. If this relation between stimuli and sensations is represented by a curve in which the ordinates express the sensations increasing by equal amounts, and the abscissas the stimuli necessary to produce them, a logarithmic curve is obtained.

There is doubtless some ground for the assumption of Fechner that least perceptible increments of sensation are equal; but nevertheless it is an assumption, and one that is made in spite of conflicting evidence. We should not, therefore, allow ourselves to be misled by the mathematical treatment of this problem in so far as it is based on an assumption the exactitude of which has not been proved. Waller (9) has pointed out that the matter is further complicated by the fact

that several steps intervene between the external stimulus and the production of the sensation. That is to say, there is first the action of the external stimulus on the end organ; then a nerve impulse in the sensory nerve fiber; and finally the process, in the brain, which gives rise to the sensation. It is a question, therefore, between which of these processes the logarithmic relationship holds good.

While sensation, for the reasons indicated, is an illusive basis for quantitative study, it is the only basis available in the higher forms. Where reactions are profoundly modified by past experience and voluntary control, it is futile to measure them to determine quantitatively the effect of the stimulus, for, instead of being expressed directly in a series of muscular reflexes, a stimulus is then translated first into sensation, and the reaction that follows the sensation may be largely determined by the past experience of the individual rather than by the strength of the stimulus. In such cases, the only thing we can measure is the sensation, or the nerve impulse that causes it.

Hence it is hardly necessary to emphasize the desirability of obtaining additional data from organisms in which the chain of events between the application of the stimulus and the resulting production of some measurable reaction, is not complicated by the factors of sensation and voluntary control based on past experience. If we can eliminate these factors, we may regard the amplitude of the reaction as an index to the intensity of the physiological processes initiated by stimulation. Such reactions are subject to exact physical measurement, and their amplitude may be plotted against the intensity of the stimulus in the form of stimulus-*reaction* curves.

It is only by a careful analysis of many such curves, based on the study of organisms having different chains of physiological processes intervening between stimulus and reaction, that we shall be able to ascertain at what point in the chain of events the approximately logarithmic character of the curves is impressed upon them. For the present it is convenient to speak of reactions presenting a logarithmic curve of increase as following the Weber-Fechner Law, or better the Weber-Fechner Curve. We should, however, be careful to avoid thinking of all the widely divergent types of reactions commonly included in this category as governed by the same law.

A review of the extensive work done on the measurement of sensations does not come within the scope of this paper. The amount of experimental work on the measurement of the amplitude of responses directly controlled by the strength of the stimulus, has been compara-

tively small. There have been many suggestions, incidental to other work, to the effect that such and such an organism, in its response to light or chemical stimulation, apparently conformed with Weber's Law; but in the majority of cases these suggestions have not been based on sufficient data to be of any great value. When therefore measurements of the light reactions of the blowfly larva, made for other purposes, (8), appeared to follow, at least approximately, the Weber-Fechner Curve, it seemed worth while to determine more carefully the exact extent of their apparent conformity with the law, and if possible the causes for it.

The problem in the case of the blowfly larva seemed particularly promising: (1) because the system of measurement, worked out in connection with previous experiments on this form, has yielded consistent quantitative data; (2) because the amplitude of the reaction was directly controlled by the intensity of the stimulus; and (3) because the stimulus reaction curve of a simple reflex of this type would appear to be of considerable value for comparison with the more familar stimulus-sensation curves of human psychology.

III. THE METHOD OF RECORDING AND MEASURING THE REACTIONS OF THE BLOWFLY LARVA TO OPPOSED LIGHTS

In these experiments, the method used for stimulating the larvae and measuring their reactions was the same as that described in det a in an earlier paper (8,pp. 223–239). It is sufficient, here. therefore, tilo outline the procedure very briefly.

The apparatus used was so constructed that the opposite sides of the animal under observation could be subjected to opposed beams of light, the actual and relative intensity of which could be varied at will. Figure 1 shows a plan of the apparatus. It was possible to control the actual intensity of the lights by varying the number of Nernst glowers thrown into circuit (fig. 1, *G*) and by varying their distance (fig. 1, *G f O*) from the observation stage. The relative intensity of the lights was controlled by moving the observation stage from a point midway between the mirrors (where the opposed beams would be of equal intensity) toward one mirror or the other. By calculating the intensities according to the law $I \propto \frac{1}{d^2}$, the distance through which it was necessary to move the stage away from the center, in order to secure any particular intensity ratio between the lights could be readily

ascertained. With the apparatus set up for the desired intensities, larvae were subjected to the stimulation of the opposed beams by forcing them to crawl into the path of light at right angles to a line connecting the mirrors.

In preliminary experiments it became apparent that there was a wide range of individual variability in the photosensitiveness of the larvae. It was not possible to eliminate this factor from comparative measurements by using a single identified larva for each series of tests, because of the shortness of their sensitive period, coupled with the rapid changes in their degrees of sensitiveness with age. The nearest approach that could be made to eliminating the effects of individual variability was to use only those larvae which, by preliminary experiment, were known to react uniformly to a standard test for sensitiveness.

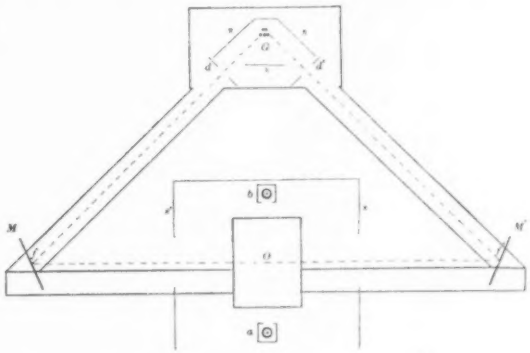

Fig. 1. Diagram of apparatus used to produce differential bilateral light stimulation. *G*, five 220-volt Nernst glowers; *M* and M′, mirrors; *O*, center of observation stage; broken lines, central rays of beams of light from the glowers reflected to *O* by the mirrors; *d* and d′, screens with rectangular openings; *s* and *s*′, light shields; *a* and *b*, 2 c.p. orienting lights with screens.

The standardizing test used consisted in subjecting larvae, which were oriented and crawling under the influence of a horizontal beam of light, to an instantaneous change of 90° in the direction of the beam. Each larva was made to crawl first toward, and then away from the observer, and thus subjected to the change of direction of the light from both its right and left sides. Those larvae orienting immediately and accurately to both changes were considered to be of "standard senstitiveness." The nature of the pairs of trails made by an animal in these tests, and the varying abruptness with which they came into orientation, is shown in figure 2. The data presented in the follow-

ing pages were all obtained from maggots thus standardized for their photosensitiveness, and they may therefore be considered reliable for comparative study.

These standardized larvae were subjected to stimulation in the following manner. Each maggot was allowed to crawl onto the observation stage immediately in front of the orienting light (fig. 1, a). Under the influence of this light it was forced to crawl toward the center of the stage in a direction at right angles to the path of light thrown by the mirrors (fig. 1 ff′). By putting a drop of dilute methylen blue on its posterior end, the larva was made to record its own trail on a sheet of paper. When the animal was well onto the stage and crawling steadily, the orienting light was turned out and the mirror

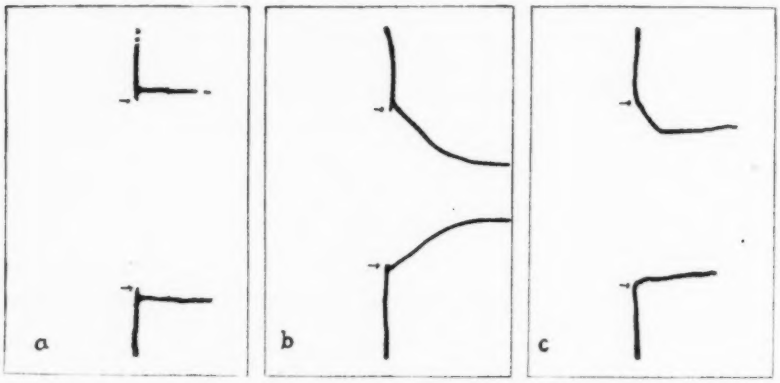

Fig. 2. Three examples of test trials which pass the standard orientation test. Each pair of trails was made by a different larva. Though there is some difference in the sharpness of orientation, each trail comes to lie parallel to the new direction of light. The arrows mark the points at which the direction of the light was changed.

beams were thrown on simultaneously from either side, the point at which the larva was subjected to the bilateral illumination being indicated on the trail. The part of the trail crawled under the influence of the bilaterally applied stimulus is the significant portion.

Loeb (5, p. 2), has pointed out that when "two sources of light of equal intensity and distance act simultaneously upon a [*negatively*] heliotropic animal, the animal puts its median plane at right angles to the line connecting the two sources of light." We should expect, then,

that a larva subjected to the action of opposed beams of equal intensity, would continue crawling in a direction at right angles to a line connecting the mirrors. That such is in fact the case was demonstarted by a large number of trials. It was, however, observed that certain larvae tended to crawl somewhat toward either their right or left even under balanced illumination. Apparently this tendency to deflect from the normal course was due to bilateral asymmetry of photosensitiveness (8). To eliminate the distorting effect of unbalanced sensitivity from the final measurements of the trails, each larva was started onto the stage first from one direction and then from the other. By thus reversing the direction of crawling and making the trails in "opposite-direction-pairs," the deflection due to

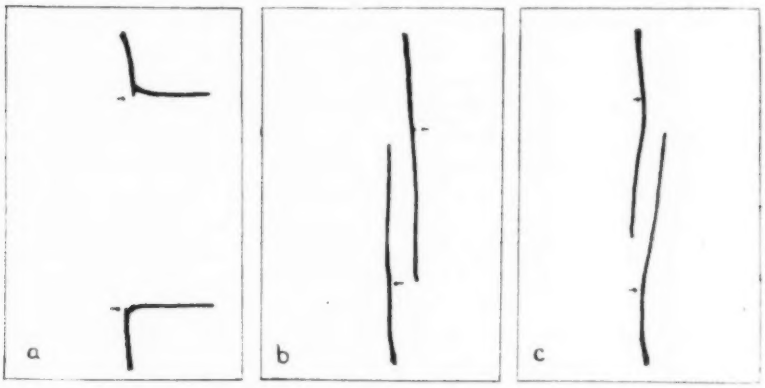

Fig. 3. A set of trails made under equal, opposed lights by a symmetrically responding larva; *a*, the test trails; *b* and *c*, trails under the influence of balanced lights.

asymmetry was thrown on opposite sides of the "norm," and would consequently be canceled out of the final measurements. Figure 3 is a photograph of the complete record of a larva's responses to equal bilateral stimulation; *a* shows the pair of trails made by the maggot in the preliminary standardizing test; *b*, an "opposite-direction-pair" of trails made under balanced illumination; and *c*, a second pair of trails similar to those in *b*, made after the larva had been rested for half an hour. In the record sheet photographed in figure 3, *c*, it will be observed that the trails do not conform exactly to the perpendicular to the line connecting the sources of light. There is a deflection to

the right in that trail made by the larva while crawling away from the observer, and to the left in that trail made by the same larva while crawling toward the observer. If now we measure the deflection of the trails in degrees, by placing a protractor with its center at that point on the trail where the head of the larva was when the lateral lights were turned on, and its base parallel to the line connecting the sources of light (fig. 1, ff′) we find that the deflections to the right and left are of equal value. By tabulating left hand deflections in *plus* degrees and right hand deflections in *minus* degrees, the effects of asymmetry are cancelled, and we have as a final result a measurement identical in value with those measurements taken on larvae that have a perfect balance of sensitiveness. As a certain amount of unbanance of sensitiveness is far more common than perfect symmetry, this checking out in all measurements of the possible effects of asymmetry is of the utmost importance. By taking the sum of the plus and minus deflections of a larva, and dividing it by the number of trails measured, the *average angular deflection* of the larva is determined. The average obtained by measuring four trails such as those shown in figure 3, *b* and *c*, expresses very accurately the response of any individual larva.

The mean of the *average angular deflections* of a number of larvae may be considered as expressing accurately the value of the response which is elicited by a given set of conditions. Table 1 is an example, taken from several similar sets of measurements that were made under equal bilateral illumination, which serves to show the method of compiling the measurements. A study of the table shows that very few trails conform exactly to the theoretical response, but the total plus and minus deflection of each larva comes near to cancelling, and the average of the four trails of each larva is close to zero degrees deflection. The average response of the whole set corresponds almost exactly to the theoretical response, the average deflection from the perpendicular being only $-0.025°$. This is astonishingly accurate when one considers that a degree on the protector of 7.6 cm. radius used in measuring the trails was only 1.5 mm.

The striking results of table 1 were confirmed by several repetitions of the experiment. The immediate significance of these results is two-fold. (1) The consistent closeness of the average trail to the perpendicular, and the equal distribution of the trails on either side of it, indicate that the method of individual measurement has eliminated from the final results the effects due to asymmetry and placed the different individuals on a uniform basis for comparison. (2) The close

conformity of the aggregate response to the theoretical response, when the lights are of equal intensity, makes a well grounded point of departure for a series of experiments with opposed lights of unequal intensities; for if there is no deflection toward either side following equal bilateral stimulation, the deflection appearing under unequal bilateral simulation may be regarded as a true expression of the physiological effect of the difference between the lights. Having, therefore, perfected a system of measuring the reactions of the larvae to light and established a fixed point of departure by the measurements under equal opposed lights, we were in a position to measure the reactions to a graded series of intensity ratios.

TABLE 1

A table giving the values of the angular deflections made by larvae under the stimulation of equal opposed lights

NUMBERS OF THE LARVAE / DATE	1ST SHEET		2ND SHEET		− TOTAL	+ TOTAL	NET	AVERAGE ANGULAR DEFLECTION
	1st trail	2nd trail	3rd trail	4th trail				
								degrees
No. 14, 13/5/'13	+10	−16	+15	− 7	23	25	+ 2	+0.50
No. 18, 13/5/'13	0	+ 6	+ 2	+ 5	0	13	+13	+3.25
No. 20, 13/5/'13	+ 7	− 8	+18	−12	20	25	+ 5	+1.25
No. 15, 13/5/'13	+25	−11	−11	− 3	22	28	+ 6	+1.50
No. 4, 13/5/'13	+ 6	− 6	+ 9	− 7	13	15	+ 2	+0.50
No. 22, 13/5/'13	0	+ 5	− 7	− 1	8	5	− 3	−0.75
No. 21, 13/5/'13	− 5	− 3	−21	+17	29	17	−12	−3.00
No. 16, 13/5/'13	+17	−14	0	− 6	20	17	− 3	−0.75
No. 11, 13/5/'13	− 9	− 1	+13	− 6	16	13	− 3	−0.75
No. 9, 13/5/'13	−11	+ 5	− 5	+ 3	16	8	− 8	−2.00
10 larvae 40 trails					167°	166°	−1°	−0.025

IV. A COMPARISON OF THE STIMULUS-REACTION GRAPHS OF THE BLOWFLY LARVA WITH THE WEBER-FECHNER CURVE

1. The graph expressing the values of the angular deflections produced by a graded series of percentage differences of intensity between opposed lights.

The problem, so far as the method of experimenting was concerned, now resolved itself into the compiling of a series of reaction measurements for comparison with various values of the acting stimulus. As

has already been pointed out, we proposed to measure, not sensations, but the amplitudes of reflexes initiated by stimuli of various degrees of intensity. The measurable reaction in the method of experimenting outlined above, is the larva's *angular deflection* from an original path of locomotion. The original path of locomotion was so arranged with reference to the lights producing the significant portion of the reaction, that the larva came into the path of the light at right angles to the line connecting their sources. We have already seen (Table 1, p. 321) that when the opposed beams were of equal intensity, there was no deflection from the initial direction of locomotion. But if we subject larvae to the action of *unequal* lights, there appears a deflection toward the weaker light, which is directly proportional to the difference of intensity between the lights (Table 3 and figure 4). We may say, therefore, that

TABLE 2

Summary of measurements of 200 trails made to determine the value of the angular deflection when the opposed lights are of equal intensity

EXPERIMENT NUMBER	NUMBER OF TRAILS MEASURED	TOTAL MINUS DEFLECTION	TOTAL PLUS DEFLECTION	NET DEFLECTION	AVERAGE ANGULAR DEFLECTION
		degrees	*degrees*	*degrees*	*degrees*
1	40	187	165	−22	−0.55
2	40	180	176	− 4	−0.10
3	40	207	225	+18	+0.45
4	40	167	166	− 1	−0.025
5	40	246	237	− 9	−0.225
Total.	200	Average angular deflection for 200 trails −0.09			

the angular deflection of the larva is made in response to a differential bilateral stimulus, the value of which may be expressed in terms of the relative intensity of the opposed lights, or more conveniently, in terms of the percentage difference between the lights.[1]

The measurements obtained from the trails of larvae subjected to equal bilateral stimulation were taken as the starting point of the stimulus-reaction curve. Table 1 shows in detail the deflection measurements taken from a set of 40 trails made by ten larvae. This set of data was supplemented by four other similar sets. Without reproducing the tables in detail, the results may be summarized as in Table 2. The totals of Table 1 appear in Table 2 as experiment 4.

[1] The percentage differences throughout the experiments are computed in terms of the *stronger* light.

Stating the results of Table 2 in terms of the series of experiments of which it forms the starting point, we may say that the reaction to opposed lights of 0 per cent intensity difference, is a minus deflection 0.09°. This is so near zero degrees that it undoubtedly signifies the absence of deflection under these conditions.

Following the experiments at equality, a series of measurements was made at the following percentage differences between the lights: $8\frac{1}{3}$ per cent, $16\frac{2}{3}$ per cent, 25 per cent, $33\frac{1}{3}$ per cent, 50 per cent, $66\frac{2}{3}$ per cent, $83\frac{1}{3}$ per cent, and 100 per cent. As the method of measurement and tabulation was the same throughout, a summarizing table will be sufficient to show the results obtained.

TABLE 3

A table based on the measurement of 2,500 trails showing the progressive increase in angular deflection with increasing differences between the lights.

Percentage Difference in Lights.	Equality 0%	$8\frac{1}{3}$%	$16\frac{2}{3}$%	25%	$33\frac{1}{3}$%	50%	$66\frac{2}{3}$%	$83\frac{1}{3}$%	100%
Number of Trails Measured.	200	200	200	500	500	500	100	100	200
Average Angular Deflection.	−0.09	−2.77°	−5.75°	−8.86°	−11.92°	−20.28°	−30.90°	−46.81°	−77.56°

These results may be expressed graphically by plotting degrees of deflection along the axis of ordinates and percentage differences of intensity along the axis of abscissas. The stimulus-reaction curve thus obtained is shown in figure 4.

It is obvious that this curve is not directly comparable with the logarithmic Weber-Fechner Curve constructed on the basis of *absolute* increments in the stimulus, since the curve of figure 4 is plotted on the basis of *percentage* increments in the stimulus. For purposes of comparison, however, a theoretical curve may be constructed of the type which would be obtained by measuring, under the conditions of these experiments, a reaction governed by the Weber-Fechner Law. Using percentage differences in stimulus instead of absolute differences, and degrees of deflection instead of sensation units, such a curve has been plotted as a broken line in figure 4.

The construction of this theoretical curve is based on the following simple deduction from the usual statement of the Weber-Fechner law. If "the increase of stimulus necessary to produce a unit increase in response is a definite fractional increment of the acting stimulus," then it follows that the same fractional increment *in a stimulus of any*

intensity will produce equal changes in response. In these experiments (see p. 322) an increase of the intensity difference between the opposed lights is regarded as an increase in the stimulus acting to deflect the larva from its original path of locomotion. For a difference of $8\frac{1}{3}$ per cent between the lights, there is experimentally a deflection of 2.77° (see fig. 4 and Table 3). Dealing in round numbers let us assume, for the plotting of the theoretical response, that a difference of $8\frac{1}{3}$ per cent

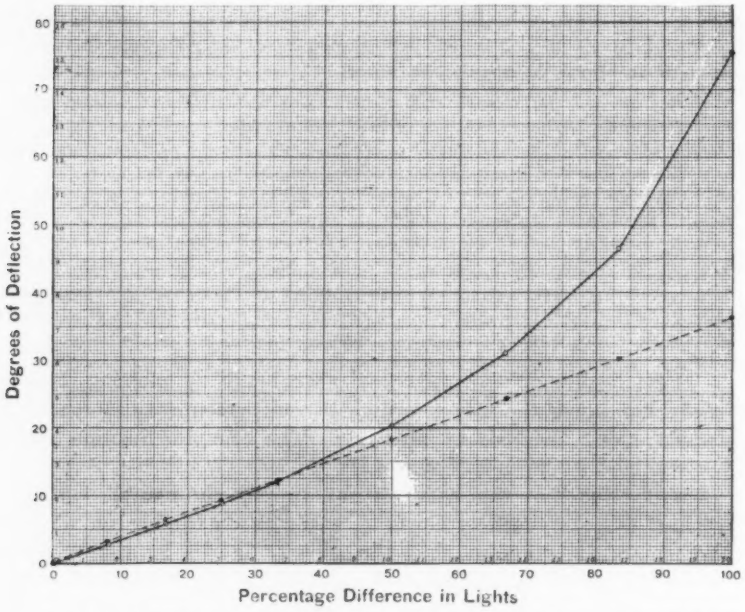

Fig. 4. The solid line is a curve representing the angular deflections of the blowfly larva under opposed lights of various intensity ratios. (See also table 3). The broken line is a theoretical curve such as would be obtained by plotting, under the same conditions of measurement, a reaction governed by the Weber-Fechner Law. In plotting this theoretical curve, a difference of $8\frac{1}{3}$ per cent between the lights is assumed to produce a deflection of 3°.

Ratio of Lights.

1 to $\frac{11}{12}$ = $8\frac{1}{3}$% difference.	1 to $\frac{2}{3}$ = $33\frac{1}{3}$% difference
1 to $\frac{5}{6}$ = $16\frac{2}{3}$% difference.	1 to $\frac{7}{12}$ = $41\frac{2}{3}$% difference.
1 to $\frac{3}{4}$ = 25% difference	1 to $\frac{1}{2}$ = 50% difference.

between the lights will produce a deflection of 3°. This point would be located in precisely the same manner as the first point on the logarithmic Weber-Fechner curve. It is after the location of the first point on figure 4 that the method of plotting this percentage-increment curve begins to differ from that of plotting the absolute-increment curve. In the former curve, each point must of necessity be figured from unity as a starting point; in the latter, only the first point is so figured; each succeeding point being based on the one immediately before it. The values of the stimuli in an absolute-increment series would run: 1 + $\frac{1}{12}$ of 1 equals 1.083; 1.083 + $\frac{1}{12}$ of 1.083 equals 1.173; 1.173 + $\frac{1}{12}$ of 1.173 equals 1.271; etc. The values in the percentage-increment series would run: 1 to $\frac{11}{12}$ ($8\frac{1}{3}$ per cent difference); 1 to $\frac{5}{6}$ ($16\frac{2}{3}$ per cent difference); 1 to $\frac{3}{4}$ (25 per cent difference); 1 to $\frac{2}{3}$ ($33\frac{1}{3}$ per cent difference); etc.; computed each time from the same starting point (unity). Each increase of $8\frac{1}{3}$ per cent in the difference between the lights is assumed, in this case, to produce an increase in deflection of three degrees. The theoretical curve plotted in this way is rectilinear (fig. 4).

The steps in this percentage-increment curve do not represent the same sort of intervals as the least perceptible increments used by Fechner. In experimental work of the type under consideration it is not practical to use least perceptible changes in reaction as units of measurement, for it involves, to too great an extent, the personal judgment of the experimentor. It is far more accurate to measure responses that are of readily appreciable amplitude by some standard unit of physical measurement. If it is desirable to compare the results thus obtained with results obtained by typical Weber-Fechner measurements, it is possible to put the two types of curves on the same mathematical basis. Suppose the Weber-Fechner unit of reaction measurement to be represented by the letter u. A response which is of considerably greater amplitude than the least detectable change in reaction may be represented as n times u. If there is justification for considering least perceptible increments as units, then we may handle $n.u$ in any formula in which u could be handled. If the Weber-Fechner unit is not reliable for quantitative work, as much experimental evidence seems to indicate, we have, by using standard units of measurement put the whole matter on a more sound quantitative basis. The curve of figure 4, though constructed on a different basis from that therefore employed in work of this nature, may be regarded as expressing the stimulus-response relationship postulated by the Weber-Fechner Law.

Because it is based only on the value of a step at the beginning of

the experimental series, the curve shown in figure 4 is not necessarily correct for the conditions of the entire set of measurements. It is, nevertheless, evident that a curve expressing the Weber-Fechner relationship plotted to percentage increase in stimulus would be rectilinear. The reason for the *apparent conformity* of the larva's stimulus–reaction curve with the theoretical curve through the range of lower intensity differences, is that the theoretical Weber-Fechner Curve was constructed on the basis of units taken from the lower portion of the experimental curve. Clearly there can be no conformity, other than a limited fortuitous one, between the theoretical rectilinear curve of the Weber-Fechner Law and the experimental stimulus-reaction curve of the blowfly larva.

2. The graphs expressing the values of the angular deflextions produced by a graded series of absolute intensities of opposed lights having their relative intensity maintained constant.

Under the experimental conditions already described, it was possible to make another series of measurements, which may be considered from the point of view of the Weber-Fechner Law.

Granting for the present Fechner's assumption that just perceptible differences in sensation may be considered as equal differences,—or sensation units,—we may make the deduction from the law already referred to on page 323, namely: if the increase of stimulus necessary to produce the least perceptible increase of response is a definite fractional increment of the acting stimulus, then it follows that, within physiological limits, a definite fractional increment of a stimulus of any intensity would produce the same change in reaction. To put it concretely, a $33\frac{1}{3}$ per cent addition to a stimulus of one unit should produce the same increment in reaction as a $33\frac{1}{3}$ per cent addition to a stimulus of five units, though the absolute increase in stimulus is $\frac{1}{3}$ of a unit and $\frac{5}{3}$ of a unit in the other.

To obtain data which could be referred to this form of the Weber-Fechner law, it was necessary to maintain the *intensity ratio* of the opposed lights constant, while the lights were varied, together, through a series of different absolute intensities. Such conditions were readily producible with the apparatus described on page 317. The intensity ratio of the two lights at any point between the mirrors (fig. 1, *M* and M′) is determined by the distances, along the path of the beams, from that point to the source of light. Therefore if the observation stage is fixed at the point where a desired intensity ratio exists, the absolute intensity of the common source of light (and with it the intensity of

the opposed beams) may be varied at will without changing the intensity ratio of the lights at the observation stage.

In these experiments the apparatus was set up for intensity ratios, corresponding to zero, $8\frac{1}{3}$, $16\frac{2}{3}$, 25, $33\frac{1}{3}$ and 50 per cent differences between the opposed lights. At each of these intensity ratios the common source of light was varied by using from one to five Nernst glowers. Table 4 gives the actual intensities of the opposed lights under the above conditions, measured at the center of the observation stage.

TABLE 4

The intensities of the opposed beams of light measured in candle meters at the center of the observation stage

PERCENTAGE DIFFERENCE IN LIGHTS	RATIO OF LIGHTS	INTENSITY OF WEAKER LIGHT IN CA. METERS					INTENSITY OF STRONGER LIGHT IN CA. METERS				
		1 glower	2 glowers	3 glowers	4 glowers	5 glowers	1 glower	2 glowers	3 glowers	4 glowers	5 glowers
Equality	1 to 1	6.32	13.9	24.6	31.5	41.1	6.32	13.9	24.6	31.5	41.1
$8\frac{1}{3}$%	11 to 12	6.05	13.4	23.6	30.2	39.3	6.61	14.6	25.7	32.9	42.9
$16\frac{2}{3}$%	5 to 6	5.77	12.8	22.5	28.8	37.5	6.93	15.3	26.9	34.5	45.0
25%	3 to 4	5.50	12.1	21.4	27.4	35.7	7.34	16.2	28.5	36.5	47.6
$33\frac{1}{3}$%	2 to 3	5.21	11.5	20.2	25.9	33.8	7.83	17.1	30.5	39.0	50.9
50%	1 to 2	4.60	10.2	17.9	22.9	29.9	9.20	20.2	35.8	45.9	59.9

The reactions of the larvae to the five different absolute intensities used at each intensity ratio of the opposed beams, were measured and the data compiled in Table 5. The same results have also been plotted

TABLE 5

A table based on the measurements of 2700 trails, showing the average angular deflections at five different absolute intensities and six relative differences of intensity

	EQUALITY	$8\frac{1}{3}$ PER CENT	$16\frac{2}{3}$ PER CENT	25 PER CENT	$33\frac{1}{3}$ PER CENT	50 PER CENT
	degree	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*
1 Glower	−0.55	−2.32	−5.27	−9.04	−11.86	−19.46
2 Glowers	−0.10	−3.05	−6.12	−8.55	−11.92	−22.28
3 Glowers	+0.45	−2.60	−5.65	−8.73	−13.15	−20.52
4 Glowers	−0.025	−2.98	−6.60	−9.66	−11.76	−19.88
5 Glowers	−0.225	−2.925	−5.125	−8.30	−10.92	−19.25
Average	−0.09	−2.77	−5.75	−8.86	−11.92	−20.28

graphically in the form of a five-point curve to express the values of the reactions obtained under each of the six intensity ratios. (fig. 5).

If the principle of the Weber-Fechner Law holds good, a definite percentage change in the value of any acting stimulus will produce the same change in reaction (see p. 326). In these experiments, therefore, the Weber-Fechner expectation is a horizontal rectinilear curve at each intensity ratio. All the experimental curves, however, show marked unevenness. Take for example the curve obtained under a $33\frac{1}{3}$ per cent difference of intensity. There is a maximum deflection of 13.15° at the three-glower intensity, and a minimum deflection of 10.92° at the five-glower intensity. The curve, however, is of a roughly hori-

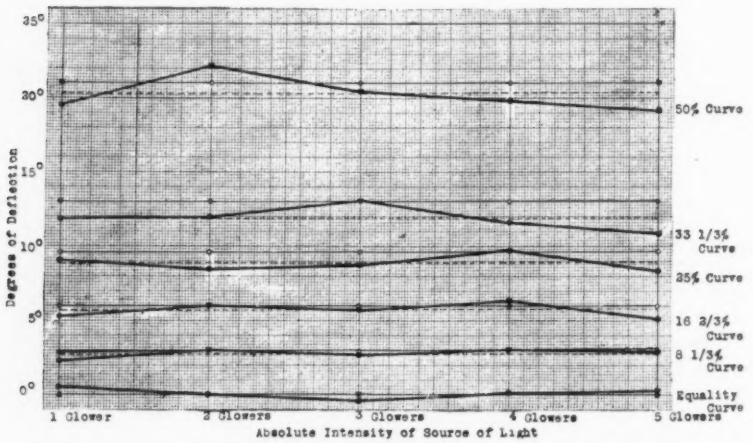

Fig. 5. Curves showing angular deflection made by blowfly larvae under opposed lights maintained at a fixed intensity ratio, while the absolute intensity was varied by using from 1 to 5 Nernst glowers at the source of light. The broken lines represent the average position of the 5 points of each curve. For significance of solid, horizontal lines see page 337.

zontal, rectilinear type. The interpretation of the results depends on how much significance we attach to the variation of 2.33° between the points of the curve most widely different in value from each other. Cases in which a moderate intensity of light, gave a greater response than a very high or very low intensity, have been observed by Walter for Planaria (10) by G. P. Adams for Allelobophora (1), etc., which made me rather expect that a similar maximum at a medium intensity might

appear in the curves run at other percentage differences. This, however, was not the case, as may be seen from the graphs of the reaction values at six different ratios of intensity (fig. 5). There is no consistency in the position of the maxima; in fact, no two curves are strikingly alike in any respect, save possibly the tendency to run low at the five-glower intensity. As the maxima depart but little from the mean, such inconsistency among the similar curves suggests that the variations are probably merely fluctuations due to experimental error. This is further indicated by computing the average position of the five intensity points of a curve and comparing the amount by which the maximum or minimum is separated from the mean, with the aver-

TABLE 6

A table showing the interrelation of percentage difference in the intensity of the the lights, average angular deflection, average error of the trails, and departure of the maximum from the mean of the curve.

RELATIVE INTENSITY OF THE LIGHTS	AVERAGE ANGULAR DEFLECTION	"AVERAGE ERROR"—AVERAGE DEVIATION OF SINGLE TRAILS FROM MEAN OF THE SET	GREATEST DEPARTURE OF ANY POINT ON CURVE FROM MEAN OF CURVE	GREATEST DEPARTURE FROM THE MEAN OF THE CURVE EXPRESSED IN % OF THE AVERAGE DEVIATION
	degrees	*degrees*	*degrees*	*per cent*
Equality	0.09	±1.98	0.53 (at 3 gl.)	27
8⅓% difference	2.77	±1.58	0.45 (at 1 gl.)	28
16⅔% difference	5.75	±1.87	0.85 (at 4 gl.)	41
25% difference	8.86	±2.93	0.80 (at 4 gl.)	27
33⅓% difference	11.92	±3.36	1.23 (at 3 gl.)	36
50% difference	20.28	±4.75	2.00 (at 2 gl.)	42

age variability of the trails used in compiling the curve. This comparison shows the departures of the maximum and minimum to lie well within the value of the "average error" of the experimental data.

Take, for example, the case of the curve which has the greatest variation, the curve at 50 per cent difference. The deflections (see Table 5 and fig. 5) are at one glower, 19.46°; two glowers, 22.28°; three glowers, 20.52°; four glowers, 19.88°; five glowers, 19.25°. The average deflection for all five intensities is 20.28°, represented on the 50 per cent curve of figure 5 by the (broken) "mean line." The greatest departture from this mean is at the two-glower intensity. Now by referring to Table 6 the average deviation of single trails from the mean trail at 50 per cent difference is seen to have been ±4.75°. The maximum

departure of this curve from the mean is but 42 per cent of the average error of the experimental data on which the curve was based. Similar computations have been made for the other curves and the figures given in Table 6. The departures of the curves from a straight line are not only well under the value of the experimental variation in all cases, but they bear a reasonably constant relationship both to the average error and to the total deflection at each intensity. As the amount of response diminishes in the successively smaller intensity differences, the average departure of the trails from the mean diminishes. And as the average error of the trails decreases, the separation of the maximum from the mean decreases correspondingly. The last bit of evidence is the variation in the curve at equality. This falls in place in the decreasing series, but yet the variations from the mean are still sufficient to indicate the fortuitous nature of the similar variations in the other curves, for if an absolutely straight line could be obtained under any conditions, it would be at equality. There seems, therefore, to be no room to doubt that the slight maxima of these curves are merely chance variations, and that the significance of each curve is a horizontal line represented by the "mean line" computed from the five points of a curve. The manner in which the curves of the series grade in together without overlapping, and the confirmation of each curve by every other curve, make the evidence practically complete.

We may therefore conclude that the curves of angular deflection values for a graded series of absolute intensities of opposed lights having their relative intensity maintained constant, resemble in contour the rectilinear curves expressing the Weber-Fechner expectation. The significance of this resemblance is a matter which is taken up at length in the subsequent discussion of the results. It is puzzling in view of the fact that the other series of measurements made on the same animal under the same experimental conditions can not in any way be regarded as following the contour of the theoretically correct Weber-Fechner Curve.

V. DISCUSSION

Apparently it is customary to consider a given reaction to be in accord with the Weber-Fechner Law, when the curve representing the magnitude of the responses produced by increasing stimulation is similar to a certain logarithmic curve said by Fechner to express the stimulus sensation relationship which Weber established for the sense of touch

in man. Astonishingly diverse types of reactions have been held to yield such a curve; the category runs all the way from the photic reactions of fungi (6) to human sensations. The heterogeneity of the reactions said to follow this law has occasioned strangely little comment. It seems hardly more justifiable to assume, without inquiring into the mechanism of the responses, that widely different organic reactions follow the same law because they exhibit similar curves relating reaction amplitude to stimulus intensity, than to classify animals on the basis of their size.

Critical consideration of the various responses said to follow Weber's Law will, I believe, lead to one of two conclusions: either there is some phenomenon fundamental to all these types of reaction which impresses its characteristics on the responses in which it occurs, whether or not they involve a central nervous system, or we have been misled by the lure of a mathematical formula and have sheltered under it a group of observations only superficially similar. Which of the conclusions is correct can be judged only in the light of further and more closely analytical work. It is not improbable that both conclusions may prove to a certain extent true. When the various reactions said to follow Weber's Law have been successfully analyzed, and when it is known *why* each reaction follows the curve that it does, it may be possible to say that a certain group of reactions follow a logarithmic course *because* there is some particular phenomenon common to them all. Quite probably the same analyses which make possible the formulation of a law governing such a group of reactions will exclude from the group certain reactions presenting a curve which is similar, but where the similarity results from causes peculiar to the individual responses. At present there is little justification for talking about a "psychophysical *law*." It can be said, only, that many reactions have been found to exhibit strikingly similar curves when increase in their amplitude is plotted against increase in stimulation. When the causes for this similarity are known, we will be in a position to formulate a law—or to abandon an old hypothesis.

The experiments dealt with in this paper were arranged to facilitate a consideration of the results from the point of view of the Weber-Fechner Law. It was hoped that if these stimulus-reaction curves did conform with the curves obtained in stimulus-sensation work, analysis of the different reactions might show a dominant factor common to both types of response. The establishing of such a factor would go a long way toward clearing up our rather hazy ideas as to the nature

of the so-called "psychophysical relationship." As we have seen, however, of the two series of measurements made on the same animal by the same experimental methods, one clearly does not accord with the Weber-Fechner Curve, whereas the other way may be regarded as following, within the limits of experimental error, the theoretic expectation of the law. If Weber's law held good for this reaction it would govern the reaction from whatever point of view the measurements were considered. Since such is not the case, we must seek some factor which is capable of producing both of the two types of curves obtained experimentally. Such a controlling factor is, I believe, demonstrable in the structural plan of the orienting mechanism of the larva.

In an earlier paper, experimental evidence was advanced which justifies the conclusion that, in this form, orientation to opposed lights depends upon symmetrically located sensitive areas operating bilaterally on the musculature (8). The evidence may be summarized as follows: (1) When the lights acting on the opposite sides of the larva are equal, the larva orients so that its median plane is at right angles to the line connecting the sources of light. (2) When the opposing lights are unequal, a deflection toward the weaker light appears. (3) Certain larvae are asymmetrical in their response, deflecting toward the less sensitive side when subjected to equal bilateral illumination. (4) The blackening of one side of the sensitive region produces a deflection toward the side thus artificially made less sensitive (3). (5) Asymmetry of sensitiveness may be balanced by a corresponding inequality of the stimuli acting on opposite sides of the animal.

In view of these facts there seems to be no explanation for the responses of the blowfly larva to opposed lights, other than the assumption that symmetrical sensitive areas affect the musculature of the two sides of the animal in proportion to the stimulation received. We do not know precisely the nature of the mechanism concerned in the reaction, but there are certain general lines on which such a mechanism must be based. "If the angle of orientation under opposed beams of light is such that the stimulation of the opposite sides is equalized, the receptive mechanism must be of such a nature that varying the axial position of the animal produces changes in the relative amount of stimulation received on opposite sensitive areas. Otherwise there would be no cause for the animal to assume a definite angle of orientation for each intensity difference between opposed beams of light" (8).

This equalization cannot be accomplished by bilaterally located sensitive areas that are parallel to each other. Change in the axial posi-

tion of an animal having localized, bilaterally placed, parallel photoreceptors, would result in a change of the intensity operating upon the sensitive surfaces of each side of the animal, but since the surfaces are parallel their projection on a plane at right angles to the rays of light would be the same whatever the axial position, and consequently the amount of light falling on the two sensitive surfaces would be always equal. If, however, we assume that the sensitive surfaces are inclined at an angle to each other, the case is different. Changes in the animal's axial position with reference to the lights will then result in changes not only of the effective illumination on both sensitive surfaces, but in the relative intensity of the light operative on the opposite photosensitive areas. It is not unjustifiable to assume as a working hypothesis, therefore, that the power to balance unequal bilateral stimulation, by a change in the position of the axis of the body, depends on the inclination to each other of the photo-sensitive surfaces.

It is possible to compute the value of an angle of sensitive surfaces which would equalize the light stimulation on the opposite sides of an animal, when the angle of orientation the animal assumes under unequal bilateral illumination is known. This has been done in the case of the blowfly larva, on the basis of angles of orientation experimentally obtained under four different intensity ratios of opposed lights.[2] The magnitudes of the four angles thus constructed are in good agreement, being 82°, 83°, 83°, and 82°. The consistency of these computations, together with the fact that some mechanism operating after the manner of sensitive areas inclined to each other at an angle seems to be the only way in which unequal bilateral stimulation can be equalized, led me to put forward the hypothesis outlined. I believe it expressed in a crude and much simplified form the dynamics of orientation in the blowfly larva.[3]

It seemed to me possible that the structure of the reacting mechanism might in this case, be the factor which determined the amplitudes of the responses to various values of the stimulus. To test this supposi-

[2] For a detailed discussion of the method of constructing the hypothetical angle, and the geometrical proof of the construction, see (8) pp. 265–268.

[3] This angle, I would emphasize again, probably does not represent the actual position of the sensitive surfaces in the larva. There are too many modifying factors intervening between the direction of the rays of light in the field and the angle of incidence of the light on the sensitive areas to permit of basing any conclusions directly on the magnitude of the computed angle, for the structural peculiarities of the dioptric apparatus, or of the tissue overlying the sensitive area would change its value to a large extent.

tion, an angle of sensitive surfaces was assumed corresponding to the value of the angle computed in the paper referred to. Taking 80° as the angle of inclination of the sensitive surfaces to each other, the angle of orientation which would equalize the stimulus bilaterally was calculated for the intensity ratios used in this seriesof experiments. The angular deflection values thus obtained were plotted graphically for comparison with the experimental curve (figure 6.) The two curves correspond so exactly that they are indistinguishable at several points.

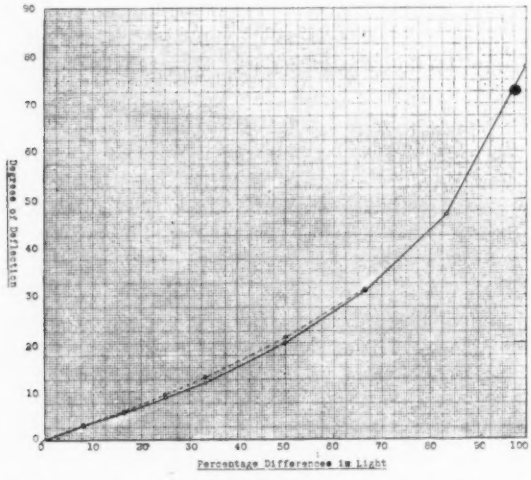

Fig. 6. A figure for the comparison of the reaction graph of the blowfly larva, with a graph showing the angular value of the axial positions that would equalize the light bilaterally in a photoreceptive system with sensitive surfaces inclined at an angle of 80° to each other. Solid line, experimental curve (see fig. 5); broken line, theoretical curve.

It will be observed, however, that the theoretical curve has not been carried beyond the $66\frac{2}{3}$ per cent intensity difference. The reasons why is not justifiable to carry the curve beyond this point are as follows. In its normal method of locomotion the blowfly larva extends its anterior end alternately to right and left, fixing its maxillal hooks in the substratum and pulling its body up by the aid of their attachment. (See also, 7, p. 176). The extent of the side-to-side swinging of the head in this process is variable and very difficult to estimate accurately.

I should place the average swinging of the lavra when not subjected to sudden change of illumination as approximately 30° to 40° on either side of the mid-position. The effect of this swinging on the amount of light intercepted by sensitive surfaces inclined toward each other at an angle of 80° is indicated in figure 7. The line AB is drawn perpendicular to the line connecting the sources of light, 1 L and 3 L. The line OC is constructed at an angle to AB (31°) such that the amount of light reaching the opposite surfaces of the 80° angle, edf, constructed about OC as a bisector shall be equal when the lights are of a three to one intensity ratio. The broken lines mx and hx are constructed at an angle of 35° to OC. They represent the axial position of the anterior end of the larva at the extreme of its swing toward either side, while the angles ghi and lmn represent the resulting positions of the sensitive surfaces. In the axial position, mx, the sensitive surface mn is shaded from the light 3 L and exposed to the light 1 L. When the angle of orientation (BOC) is not great enough for the side-to-side swings to shade one sensitive area, they would not appreciably influence orientation, because the swings being equal in extent toward either side, the total effect of the light would be approximately equivalent to its effect in the average position ($< f\,d\,e$ fig. 7). The particular angle of orientation here illustrated lies at about the critical point where the side-to-side swinging begins to alter the simple action of the lights which holds at smaller angles of orientation. Although in this case the extent of the arc of swinging in which the effect of the shading is felt is so small that it has little influence on the angle of orientation, yet at the wider angles of orientation the effect of the shading becomes rapidly greater, causing the slope of the curve to change markedly

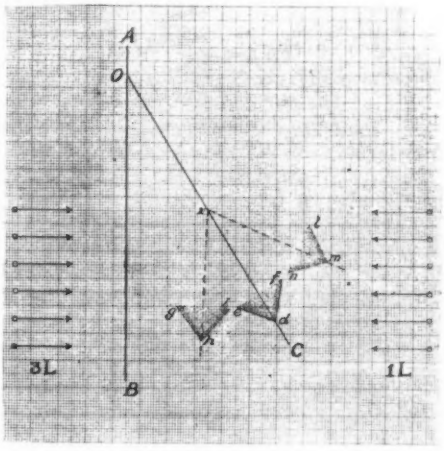

Fig. 7. For explanation see text, pages 334 and 335.

in the region where this second factor first becomes operative. (See fig. 6, experimental curve.)

Calculations have been made for the upper portion of the reaction graph on the basis of the light energy intercepted by both surfaces in the complete arc of swinging. This treatment, however, is of too uncertain a nature to be of any quantitative value. Still it yields results which, in a general way, accord with the more abrupt rise in the upper portion of the experimental curve.

We would seem, therefore, to be justified in stating that an animal possessing the peculiarities of locomotion characteristic of the blowfly larva, together with bilaterally located photosensitive surfaces inclined toward each other at an angle of 80°, which influence the musculature of the opposite sides in proportion to the stimulation they receive, would, if subjected to the conditions of these experiments, yield a type of reaction graph similar to that plotted from the measurements made on the blowfly larva.

The type of curve which would be produced under the conditions of the second set of experiments (see pp. 326–330) by a mechanism of the nature postulated is much more readily ascertainable. It will be recalled that the stimulus there employed was "a graded series of absolute intensities of opposed lights having their relative intensity maintained constant." Suppose that under an intensity ratio of three to one between the opposed lights, the axial position of the animal is changed until it attains an angle of orientation such that the amount of light falling on the opposite sensitive surfaces is equal. The position of the photoreceptive system would be that illustrated by the angle *e d f* in figure 7. As already pointed out, this equalization depends on the fact that the sensitive surface toward the light of three units intensity, (3 *L*) presents to the rays an area of one-third that presented by the symmetrical sensitive surface of the opposite side of the animal to the light of one unit intensity (1 *L*). Whatever change is made in the absolute intensities of the opposed lights, as long as this ratio is maintained, *the same axial position will equalize the stimulus bilaterally.*

We should obtain, therefore, for any intensities of fixed ratio to each other, horizontal, rectilinear reaction curves. The positions of the graphs for the conditions of these experiments were determined theoretically when the angles of orientation which would equalize the light at given intensity ratios were computed (see fig. 6 and p. 334). We therefore know the contour and position of the reaction graphs which would be produced under these conditions by the action of the mechan-

ism postulated. These curves plotted for comparison with the experimental curves of figure 5, fall in the position of the solid, horizontal lines.

By computing the average variability of the experimental data and comparing it with the values of the fluctuation of the curves, the conclusion was reached (p. 330) "that the significance of each curve is a horizontal line represented by the 'mean line' computed from the five points of a curve." The close conformity of the experimental and the theoretical curves is so striking that it needs no further emphasis.

Conclusions from such conformity as has been demonstrated above, must be drawn with extreme caution. The fact that a mechanism such as postulated would produce both types of curves obtained experimentally is in no wise to be regarded as proof of the existence of precisely such a mechanism in the blowfly larva. It does, however, present a hypothesis on which further work may profitably be undertaken. Moreover such a hypothesis offers a logical explanation of the contour of a group of stimulus-reaction graphs for which there is no other explanation available.

The most interesting phase of the results as far as the Weber-Fechner Law is concerned is the conformity with its theoretic expectation shown by the curves of figure 5. This agreement might easily have led to entire misinterpretation of the results, had not a second set of measurements been made which was clearly unconformable with the law. As both series of data were obtained under identical experimental conditions the conclusion is unavoidable that the conformity with Weber's Law, where it does occur, is entirely accidental. This does not necessarily signify that a following of the Weber-Fechner Law could not be demonstrated in the photic reactions of the blowfly larva, but that the nature of the reaction mechanism involved in the response to opposed lights is such that it dominates the other factors involved in the reaction. It is entirely possible that experiments made in such a way as to exclude the action of this mechanism for obtaining a bilateral balance of stimulation, might yield results in accord with the Weber-Fechner Curve.

VI. SUMMARY AND CONCLUSIONS

We have in this paper dealt with experiments devised for measuring the reactions of the blowfly larva so that they might be employed for comparison with various values of the acting stimulus. The measurements were so made and collected that they could be handled from

the same point of view as the available data on the comparison of sensation value with stimulus intensity. Analysis of the results obtained, with reference to the Weber-Fechner Law, brought out the following conclusions:

1. The graph expressing the values of the angular deflections of the blowfly larva from an original path of locomotion at right angles to the line connecting the sources of light, produced by a graded series of percentage differences of intensity between opposed lights cannot be regarded as conforming to the Weber-Fechner Curve.

2. Graphs expressing the values of the angular deflections produced by a graded series of absolute intensities of opposed lights having their relative intensity maintained constant, may be regarded as in conformity with the Weber-Fechner expectation.

3. The fact that two sets of data derived from the same animals, by identical experimental procedure, yield opposite conclusions so far as the Weber-Fechner Law is concerned, indicates that the agreement with the law, where it does occur, is a purely fortuitous one, and that the reactions of the blowfly larva to opposed lights are controlled by other factors.

4. It is possible to demonstrate geometrically that a photoreceptive mechanism operating after the manner of bilaterally located sensitive surfaces inclined at an angle to each other and affecting the opposite sides of the musculature in proportion to the stimulus they receive, would determine *both* types of curves which have been plotted from experimental measurements of the photic reactions of the blowfly larva.

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THE INFLUENCE OF THE EXTRACT OF THE POSTERIOR LOBE OF THE HYPOPHYSIS UPON THE SECRETION OF SALIVA

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Of the glands of internal secretion, probably one of the least understood, though one of the more important is the hypophysis, least understood, because of its inaccessibility and its seeming paradox in that the anterior lobe is the vital portion, though its extract is physiologically inactive, while the posterior lobe, although not essential to life, furnishes an extract with marked physiological properties; important, because of the changes associated with its pathology. The action of the extract of the posterior portion of the pituitary body on the circulatory system has been fairly well established, but its effect on secretion is still in dispute. Schäfer and Herring (1) concluded that it has a specific action on the renal epithelium, while Houghton and Merrill (2) maintained that the diuresis observed is due to vascular changes. Hallion and Carrion (3) noted a vasodilation following the primary vasoconstriction of the kidney, and also that the diuresis followed this vasodilation. Their results were verified by King and Stoland (4) who were unable to obtain diuresis except during vasodilation in the kidney. Frankl-Hochwart and Frölich (5) asserted that pituitary extract has no influence on the chorda tympani nerve nor on any of the secretory nerves to the salivary glands. Pemberton and Sweet (6) found that pituitary extract inhibits the secretion of pancreatic juice, supposedly by action on the cells. Edwards (6) maintains that no inhibition occurs except what can be accounted for by the vasoconstriction. In view of these results it would seem that ample opportunity was offered for further work, and at the suggestion of our instructor, Dr. O. O. Stoland, we decided to conduct a series of experiments to determine, if possible, the effect of pituitrin upon the secretion of saliva, and whether this effect is due to vascular changes in the organ or to a specific action on the cells or the secretory nerves.

On account of its accessibility and the fact that it has definite secretory and vasodilator fibers from the chorda tympani and vasoconstrictor and secretory fibers from the sympathetic, the submaxillary gland seemed to lend itself more readily than any other organ to our work. It appeared to us that the results obtained with this gland could be more readily controlled and checked. The work done by Mathews (7) on the effect of shutting off the blood supply to the submaxillary gland was of prime importance to us, in that his results show that the function of the gland was not impaired by shutting off the blood supply, but that with restoration of circulation the flow of saliva returned. He also showed that saliva would flow for almost a minute after the blood supply was shut off. From this we may conclude that a mere decrease in blood supply would not be sufficient to cause an almost immediate cessation of salivary flow.

The experiments reported in this paper were carried out on healthy, well-nourished dogs, varying from 4 to 20 kilos in weight. The animals had no special preparation except that they were not fed on the day of experimentation. Ether was used as the anaesthetic throughout and was so administered by a tracheal cannula that a relatively constant degree of anaesthesia was attained by the time injections were to be made. Wharton's duct was isolated, usually on the left side, and a glass cannula inserted. The blood pressure changes were recorded by a mercury manometer connected with the right carotid artery. The external jugular vein and its branches were dissected out to expose the venous return from the gland. In most cases this consisted of a branch from the upper and anterior portion of the gland to the external jugular just before it passes through the capsule of the parotid gland and a branch from the posterior portion of the gland to the external jugular or to a branch of the latter. Variations consisted in having only one of these, most commonly the anterior branch. All branches of the external jugular except those coming from the gland were ligated, and a cannula, well paraffined and oiled, was inserted into the external jugular vein, and the blood flow through the gland recorded by the drop method. The flow of saliva from the gland was recorded by the same method. All injections were made into the femoral vein towards the heart. Continuous tracings were taken of the blood flow and salivary flow from the gland and the general blood pressure except in the first few experiments where the flow of saliva and blood pressure alone were recorded. These experiments were performed as a control against later experiments where longer

exposure of the gland was necessary. We found that exposure did not alter the results. The pituitrin made by Parke, Davis and Company was used exclusively in our work. After the animal was ready for injection, we usually found that the flow of saliva was very meager, and in order to stimulate a flow one of two methods were resorted to: the chorda tympani nerve was stimulated peripherally by a weak faradic current, or the flow was augmented by the injection of small amounts of pilocarpine. When pilocarpine was used, pituitrin was not, as a rule, injected until the blood pressure had again returned to normal. In order to control our results, variations in procedure were frequent, and the results will be given in the order in which the experiments were performed, as far as possible. The same procedure was followed until consistent results had been obtained. One cubic centimeter of pituitrin was injected unless otherwise stated.

Throughout all our work on dogs one fact, about which there could be no reasonable doubt or question because there was no exception, stood out prominently, namely, that the intravenous injection of pituitary extract invariably caused a marked decrease in the flow of saliva from the submaxillary gland. From all our experiments we found that the average slowing of saliva due to the action of pituitrin was 82.4 per cent in 47 injections, and that of these in only 13 was there a slowing of less than 75 per cent. In many cases the flow of saliva ceased altogether for a short time. In some animals more than one injection of pituitrin was made, but we failed to find in any of these cases that repeated injections decreased its effect at all except in one experiment where a large cat was used: In this case we found that the slowing of saliva was decreased after the second injection of pituitrin, and that there was hardly any slowing after the third. In all our work, also, an accompanying diminution of the blood flow from the gland was noted following every injection of pituitrin. Now the question arose, was this great decrease in salivary flow due to changes in blood supply to the gland, associated with the vasoconstriction caused by pituitrin, or to its effect upon the secretory nerves or gland cells of the organ, or to a combination of both of these? In order to analyse the causes of this great diminution in salivary secretion, we averaged the results obtained on the injection of pituitary extract during the stimulation of the chorda tympani nerve, during the action of pilocarpine, during the action of adrenalin, and during the action of chrysotoxin. The results are recorded in Table 1.

A series of three experiments was performed where the injection

TABLE 1

TREATMENT BEFORE AND DURING THE PITUITRIN ACTION	NO. OF ANIMALS	BEFORE THE INJECTION OF PITUITRIN		AFTER THE INJECTION OF 1 CC. OF PITUITRIN		
		Blood (drops per min.)	Saliva (drops per min.)	No. of injections	Blood	Saliva
Faradization of chorda tympani	3	84	21	6	30.5	4.6
Pilocarpine stimulation	13	58.6	13.7	16	16	2.57
Adrenalin stimulation	4	21.6	5.3	4	9.8	1.5
Action of chrysotoxin	7	48.2	5	7	15.5	0.35

of pituitrin was made during the faradization of the chorda tympani. Here it was observed that the slowing of saliva was 80 per cent in six injections, while the blood flow decreased only 64 per cent. We found that in most cases the blood flow almost stopped momentarily, but later the flow increased greatly while the salivary flow continued decreasing (see fig. 1). This would indicate that pituitrin acts upon

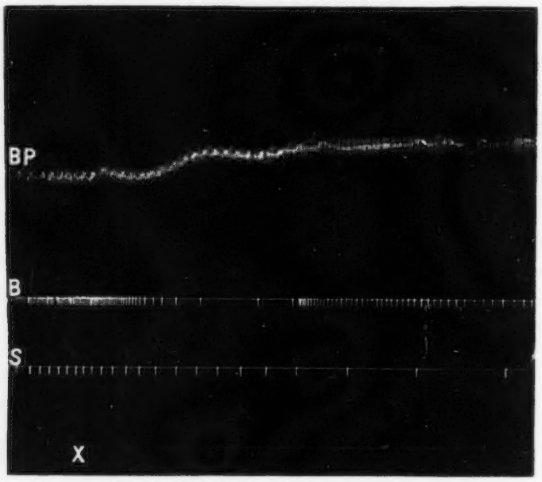

Fig. 1. The effect of pituitrin upon the flow of saliva and blood from the submaxillary gland during light faradization of the chorda tympani. *BP*, blood pressure; *B*, drops of blood from the venous return of the submaxillary gland; *S*, saliva from Wharton's duct in drops; *X*, 1 cc. of pituitrin injected.

the secretory mechanism of the gland by some other method than by vasoconstriction alone, probably by blocking or decreasing the irritability of the secretory fibers, for, as was noted above, the salivary secretion slowed even when the blood flow was fairly rapid.

We observed that during pilocarpine stimulation the slowing of the salivary flow, following the injection of pituitrin, was equal to that obtained during chorda stimulation, but that while the decrease in the blood flow during pilocarpine action was 73 per cent (see fig. 2), that during faradization of the chorda was 64 per cent. As is well known, pilocarpine stimulates the endings of the secretory nerves, while excitation of the chorda tympani affects both the vasodilator

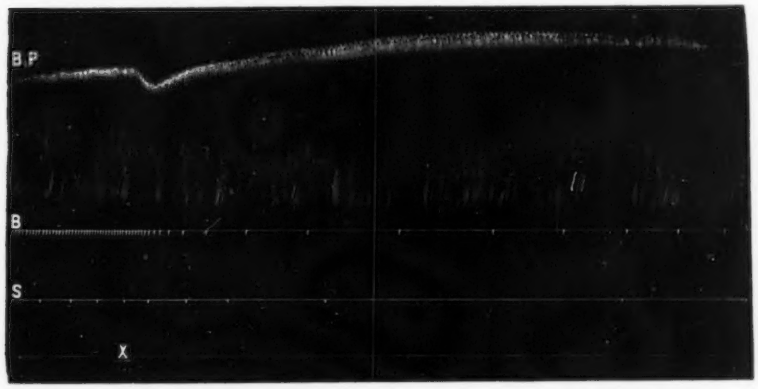

Fig. 2. The effect of pituitrin upon the flow of saliva and blood from the submaxillary gland during the action of pilocarpine. The letters are same as in figure 1.

and secretory nerves, and hence the decrease in blood flow, due to the action of pituitrin, would not be so marked in the latter case. Some increase in blood flow was noted on stimulation with pilocarpine, but was evidently the result of the greater activity of the gland rather than to any action of this drug upon the vasodilators. From this we may assume that pilocarpine acts in the same way upon the submaxillary gland as does faradization of the chorda, with the added advantage that in the former case the vasodilators are probably not affected. Now the fact that the slowing of saliva following the action of pituitrin was the same under two different kinds of stimulation, both involving the secretory fibers, coupled with the fact that the accompanying

decrease in venous return was not the same because the vasodilators were probably not stimulated to the same extent in one case, would seem to indicate that this drug inhibits in some way the secretory fibers to the gland.

Another result obtained with pilocarpine, seemingly the opposite of the normal, was the great rise in blood pressure following the injection of this drug during the action of pituitrin. Since pilocarpine normally causes a slowing of the heart and a fall in blood pressure, we are at a loss for a satisfactory explanation of this apparent paradox. The heart beat was slowed, but its amplitude was in some cases markedly increased.

TABLE 2

The results recorded in this table show the effect of pilocarpine upon the flow of blood and saliva before and during the action of pituitrin and the effect of adrenalin upon the same under like conditions.

INJECTION	NO. OF ANIMALS	NO. OF INJECTIONS	BLOOD (DROPS PER MIN.)	SALIVA (DROPS PER MIN.)
Pilocarpine	5	7	59.5	24
Pituitrin	5	7	15.4	2.1
Pilocarpine during action of pituitrin	5	7	20.5	3.2
Before adrenalin	4	4	31	2
Adrenalin	4	4	49	5.5
Pituitrin before injection of adrenalin	7	8	12	2.8
Adrenalin following injection of pitruitrin	7	8	17.7	1.5

We found further that pilocarpine was relatively inactive during the action of pituitrin. In this series of experiments (see Table 2) an injection of pilocarpine was made to stimulate a rapid flow of saliva, followed by an injection of 1 cc. of pituitrin, and at various stages of pituitrin action a second injection of pilocarpine was made. Table 2 shows that although the second injection of pilocarpine causes a slight increase in blood and salivary flow during the action of pituitrin, it is relatively inactive, because the increase can be accounted for by the partial recovery from the action of the pituitrin. In some of our experiments it was noticed that there was no increase in the flow of saliva, even though the blood flow increased a little. This would indicate that pilocarpine may have some action on the vasodilators, or more

likely that the activity of the gland was increased without apparent increase in the salivary flow, possibly by increasing the organic constituents. Since we made no quantitative determination of organic matter in the saliva, we are unable to do more than merely to state this possible explanation. In one experiment, where two successive injections of pituitrin were made to stop the flow of saliva, the real effect of pituitrin was probably shown better than in any other of this series. During this action of the hypophyseal extract it was observed that though the flow of blood was doubled after the first injection of pilocarpine, the flow of saliva remained the same, and when the second injection of pilocarpine was made eight minutes later, even then the saliva increased in less measure than the flow of blood, which is contrary to the normal action of pilocarpine. At this time the action of pituitrin should have been fairly well spent, for the blood pressure had almost returned to normal. The above observations would seem to show that the action of pituitrin is partly, at any rate, upon the secretory fibers to the gland.

In a series of four experiments we attempted to show the effect of shutting off the blood supply on the flow of saliva normally and after the injection of pituitrin. Normally the salivary flow ceased in less than one minute after shutting off the blood supply, and the same result was obtained after injection of pituitrin, the pituitrin being injected half a minute before the blood supply was shut off. This procedure failed to give any definite results. The only effect noted was that the blood flow returned slightly more slowly, and the saliva flow did not return for one minute, while normally both returned together. This is borne out by the work of Mathews (7) who shut off the blood supply for long periods of time (7 to 30 minutes).

In our experiments the blood supply was shut off for one minute. In one case the pituitrin was injected while the blood supply was shut off for 1.25 minutes and when the circulation was restored, the flow of saliva and blood both returned immediately but later slowed due to pituitrin in the blood. This is of interest in so far that it shows the peripheral action of pituitrin, because the nervous mechanism of the gland was intact. Aside from this, shutting off the blood supply furnished no other evidence than that we had already obtained by other methods, and therefore it was abandoned.

Comparing the average percentage of slowing of blood and saliva in 33 injections of pituitrin (see Table 1), we noted that the flow of saliva decreased 81.8 per cent, while the slowing of blood was 65 per

cent. This in itself would point to the probability that the decrease is not due entirely to vasoconstriction but to some other factor also. Can it be attributed to the inhibitory effect on the secretory fibers or to the toxic effect on the gland? The latter would be ruled out by the general lack of toxicity of pituitrin. This leaves us the hypothesis that the inhibition of secretion is practically due to the action on the secretory fibers to the gland, especially in view of the ineffectiveness of pilocarpine during the action of pituitrin.

In order to obtain further evidence as to the influence of pituitrin upon secretion, it was concluded to try the effect of epinephrin, the great general vasoconstrictor, which acts on the endings of the vasomotor nerves. However, we found that this drug invariably caused a vasodilation in the submaxillary gland, while effecting a general vasoconstriction. Epinephrin then presumably stimulates the vasodilator endings in this gland. Epinephrin also increased the flow of saliva; in some cases we used it to start the secretion (see Table 1). In four injections where adrenalin was used to initiate the secretion, the salivary flow decreased 72 per cent, while the blood flow decreased 55 per cent after the injection of pituitrin, thus practically paralleling the results obtained during faradization of the chorda tympani (see Table 1), a fact which would support the hypothesis that pituitrin acts upon the secretory fibers.

From Table 2, we note that when epinephrin was injected during the action of pituitrin, it caused an increase in the flow of blood, but a decrease in the flow of saliva (see fig. 3). This diminution in salivary secretion during vasodilation in the gland must be due to the increased amount of pituitrinized blood passing through the gland on account of the action of epinephrin, thus augmenting the effect on the secretory fibers. As far as we can see, the above evidence alone would go far to prove our case.

In the hope of abolishing or decreasing the action of the vasoconstrictors to the gland, we made injections of Jacobi's chrysotoxin (8) and followed this by injections of epinephrin and pituitrin. This chrysotoxin was made by extracting powdered ergot with ether, evaporating off the ether, and precipitating the oily extract with light petroleum. The precipitate was dissolved in physiological salt solution and then injected. No effect was noted on blood pressure or salivary flow, but with one preparation of chrysotoxin especially we found a marked increase in the amount of venous return from the gland, which may be attributed to the depression of the tone of the vasoconstrictors

to the gland. The injection of adrenalin during the action of this ergot preparation caused only a slight primary rise in blood pressure, followed by a fall, or in some cases there was no change at all in the blood pressure noted; in one case we got "vasomotor reversal." The flow of blood and saliva after adrenalin was unaffected to any marked degree, though in some instances a slight slowing of both was observed during the primary rise.

A further study with the method of preparation and properties of the ergot preparations since the completion of our experiments has led us to believe that the material used in our work was somewhat lacking in strength and purity; its action, however, was sufficient for our in-

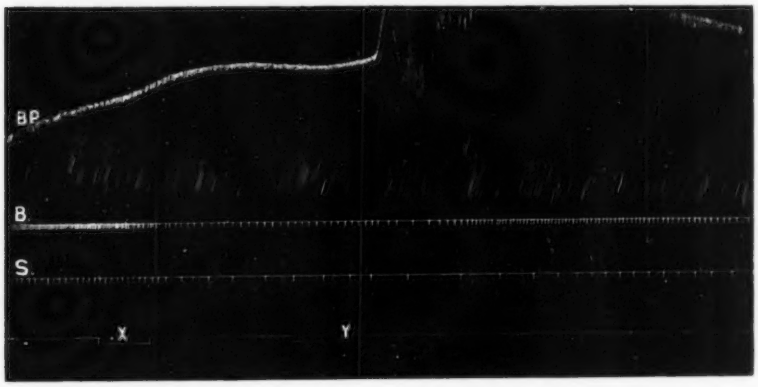

Fig. 3. The effect of epinephrin upon the flow of saliva and blood during the action of pituitrin. The letters are same as in figure 1; *Y*, injection of 2 cc. 1–50,000 epinephrin.

tentions. Having found that chrysotoxin inhibited the action of the vascoconstrictors to a marked degree, we were ready to try the effect of pituitrin during the action of chrysotoxin. We found that normally the vasoconstriction after injection of pituitrin was almost invariably preceded by at least a slight fall. When pituitrin was injected during the action of chrysotoxin we observed that the primary fall in blood pressure was, as a rule, greater than normal, while the secondary rise, though characteristic, was in most cases less marked. It was noted that the flow of saliva diminished before the decrease in blood flow was noticeable. In all but two cases the salivary slowing was noted while there was an increased output of blood from the gland (fig. 4). Dur-

ing the action of this drug the vasoconstriction after pituitrin was much less marked in most cases, the blood flowing fairly rapidly, while the decrease in flow of saliva was as great as or greater than that obtained in our other experiments.

The results obtained with chrysotoxin constitute our strongest evidence that pituitrin acts by inhibiting the secretory fibers. A number of workers have attributed the vasoconstriction caused by pituitrin to a direct action on the muscles of the arterioles; our experiments with chrysotoxin would indicate that whatever the true seat

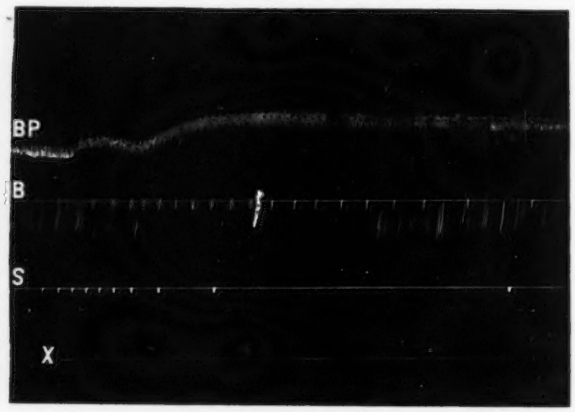

Fig. 4. The effect of pituitrin upon the flow of saliva and blood from the submaxillary gland during the action of chrysotoxin. The letters are same as in figure 1. The chrysotoxin was injected about 2 minutes before the injection of pituitrin and the dose had previously been shown to be sufficient to depress the irritability of the vasoconstrictors.

of action may be, this substance depresses the sensibility of the mechanism affected by pituitrin. Pituitrin probably acts upon the endings of the vasomotor nerves as well as upon the involuntary muscles.

SUMMARY AND CONCLUSION

1. Pituitrin invariably caused a diminution in flow of blood and saliva from the submaxillary gland, as shown from results obtained from 30 dogs and 1 cat.

2. The decrease in flow of saliva was greater than the accompanying decrease in blood flow.

3. The slowing of blood was less marked if the injection was made during faradization of the chorda tympani than during pilocarpine-stimulation, while the slowing of saliva was the same.

4. Pilocarpine was relatively ineffective even when injected seven or eight minutes after pituitrin.

5. While epinephrin normally caused a vasodilation of the gland and increase in salivary secretion, epinephrin during the action of pituitrin had the normal effect on the blood flow but caused a diminution in salivary flow, probably due to the greater quantity of pituitrin coming in contact with the gland.

6. When pituitrin was injected during the action of chrysotoxin, the decrease in the flow of saliva set in before the vasoconstriction in the gland occurred. In five out of seven cases, the flow of saliva slowed while there was active vasodilation in the gland.

From these results we must conclude that the decrease in flow of saliva following the injection of pituitrin is due to inhibition of the action of the secretory nerves to the submaxillary gland, but also due in part to the accompanying vasoconstriction, which is caused by direct action on the muscles of the arterioles or the effect on the peripheral endings of the vasomotor nerves, but more probably to the effect on both. The decrease in output of blood from the gland may be also due to the decreased activity of the gland.

We wish to express our thanks to Dr. O. O. Stoland for his valuable suggestions and criticisms in performing this series of experiments.

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THE INFLUENCE OF THE VAGUS NERVE ON THE GASEOUS METABOLISM OF THE KIDNEY

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Asher and Pearce (1) have reported a series of experiments the results of which they interpret as demonstrating the presence of renal secretory fibers in the vagus nerve. In these experiments, which were done on decerebrate animals, the urine obtained during a period of vagus stimulation exceeded in amount that obtained during a normal period of equal duration. The technical difficulties of the operation made it impossible to carry out more than a few successful experiments, so that a relatively small number of results are reported.

To secure additional data in this connection is the object of the present research, and for this purpose we have not depended on the rate of urine formation, but have sought for evidence of changes in the functional activities of the renal cells. To do this we have taken advantage of the fact that every increase in tissue activity is accompanied or followed by an increase in oxygen consumption. Barcroft (2) and his co-workers have established such a relation in the case of the kidney, their researches having shown that two types of diuretics may be distinguished: one whose diuretic action is accompanied and the other unaccompanied by an increase of oxygen consumption. It may be considered that the action of the former type of diuretics (urea, caffein and sodium sulphate), is due primarily to an actual stimulation of the excreting cells of the kidney, while the action of the latter type (sodium chloride, etc.) depends on an alteration in the osmotic pressure of the blood, produced by the injection of these salts into the body. This relationship being established, it follows that an estimation of the oxygen absorption of the kidney will be an index of the activity of the renal cells under varying conditions.

The diuresis produced by vagus stimulation must be a result of increased renal activity and for two reasons: viz., 1st, it is impossible for a nerve to change directly the osmotic pressure of the blood; 2nd,

the blood flow through the kidney does not become altered under the conditions of the above described experiment (3).

An increase in oxygen consumption by the kidney should accompany the stimulation of the vagus if this nerve really contains renal secretory fibers. To test this an estimation was made of the oxygen intake of the kidney under the conditions of Asher and Pearce's experiment; that is to say, the percentage difference between the oxygen af the arterial and venous bloods was compared. The reflex disturbances arising from the manipulation of the kidney or ureters is discounted by comparing the changes occurring in the stimulated kidney with those obtained in the opposite kidney, which was previously denervated. The fact that the vagus does not influence the circulation of the kidney (3) makes the estimation of the volumes of the blood flow unnecessary in measuring the oxygen consumption, and makes it possible to determine this by comparing the percentage amounts of oxygen in the arterial and venous bloods before and during vagus stimulation.

METHODS

The preparation of the animal conformed in all essentials to that followed by Asher and Pearce. Dogs were etherized and quickly decerebrated. The vasomotor control exercised on the renal blood vessels through the splanchnic nerves was removed on the left side by cutting the nerve just above the adrenal gland. The kidney on the opposite side (right) was at the same time completely removed from the nervous system by severing the nerves surrounding the renal vessels and searing the vessels with concentrated phenol solution. Electrodes were placed on the vagi below the level of the heart, and the nerves cut in the neck. The viscera were retracted and held in such a manner as to expose the renal veins on both sides. Samples of 1 cc. each of blood were removed from the renal veins and from the carotid artery through fine needles into hypodermic syringes containing $\frac{2}{10}$ cc. of hirudin dissolved in boiled Ringer's solution. After removing the blood, the syringe was sealed by sucking up a small quantity of mercury and capping the end, from which the needle had been detached, with a closed rubber tube. The oxygen in the various samples was estimated by the so-called differential method as described by Barcroft for determining the total oxygen in $\frac{1}{10}$ cc. of unsaturated blood (4). Artificial respiration was maintained throughout the experiments and every precaution was taken to keep the blood pressure at a constant level.

In most of the experiments this was accomplished fairly well. Twenty-five to thirty minutes elapsed between the decerebration and the taking of the blood samples. The possible disturbing influence of anaesthesia was thus eliminated.

The procedure followed in obtaining the samples of blood was the same in all the experiments. The first samples of blood were removed from the renal veins of both kidneys and from the carotid artery, and were used to determine the normal gas exchange. Immediately afterwards the vagi were stimulated with a current of approximately the same strength as that used by Asher and Pearce, for alternating periods of one minute with one minute intervals between. During the fourth minute of stimulation the second samples of blood were removed as above described and were used to determine the exchange occurring during stimulation.

The oxygen percentages of the venous bloods were compared with those of the arterial bloods of the corresponding periods, and the number of cubic centimeters of oxygen absorbed per cubic centimeter of blood calculated in each case. The right kidney, since its nerves had been destroyed, could not be affected directly by stimulation of the vagus nerve. Its oxygen consumption should therefore be constant, or if any change was found in its oxygen intake, it must be sought in some systemic condition which would effect the left kidney in the same degree.

EXPERIMENTAL RESULTS

In the accompanying table the results of the last four experiments are tabulated. The figures in the third column give the oxygen content of the arterial blood, and those in the fourth and fifth columns the oxygen content of the venous blood of either kidney before and during a period of vagus stimulation. The sixth and seventh columns give the actual cubic centimeter of oxygen taken from 1 cc. of blood by either kidney, and the last two columns, the percentile change in oxygen consumption which accompanies the stimulation of the vagus.

Experiment No. 5 is the only one of the series in which there is a greater amount of oxygen used during a period of stimulation of the vagus than can be attributed to systemic conditions as reflected in the secretion of the right denervated kidney. In all of the remaining experiments the change in oxygen consumption in the left kidney accompany-

ing vagus stimulation is less than that found in the case of the denervated right kidney.

The operative procedure of these experiments, as in those of Asher and Pearce, is very severe, and it is difficult to maintain the animal in an unchanging condition in regard to the circulation, respiration and renal function. The tables show that in spite of pains taken to maintain the normal respiratory function, there was a marked decrease in the oxygen content of the arterial blood during the stimulation of the vagus. The exact cause of this change is not at present clear. The actual oxygen consumed by each kidney, however, remained practically constant, as shown by the figures in columns six and seven of the table.

In the experiments reported by Barcroft and Brodie, in which the effects of certain diuretics on the urine flow and oxygen consumption of the kidney were investigated, an increase in urine excretion was accompanied by a like change in oxygen used by the kidney. These investigators were unable to estimate the actual amount of oxygen necessary for the excretion of a fixed amount of urine, but the experiments show that it is of such magnitude that the oxygen intake can be made a test of the physiological activity of the kidney.

The failure to obtain evidence of an increased gaseous exchange following stimulation of the vagus nerve under the conditions reputed to produce a diuresis, as in the above series of experiments, can be interpreted by assuming, either that the diuretic effect failed to appear, or that it occurred independently of any energy change in the kidney. It is to be regretted that the experimental procedure is such that simultaneous investigations of the gaseous exchange and urinary outflow are not practicable. It is, however, reasonable to suppose that in a series of eight experiments, some would show a change in oxygen consumption if the vagus nerve exerts any marked secretory influence on the kidney.

SUMMARY

The oxygen consumption of the left kidney before and during the stimulation of the vagus below the level of the heart and after section of the left splanchnic nerve, remains unchanged. This offers evidence against the supposed existence of renal secretory fibers in the vagus nerve.

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TABLE 1

EXPERIMENT PERIOD		CC. OF OXYGEN PER 1 CC. OF BLOOD			CC. OF OXYGEN CONSUMPTION PER 1 CC. OF BLOOD		PERCENTILE CHANGE IN OXYGEN CONSUMPTION DURING PERIOD OF VAGUS STIM.		REMARKS
		Carotid blood	Right kidney (denervated)	Left kidney (experimental)	Right kidney	Left kidney	Right kidney	Left kidney	
5 April 8	Normal	0.2035	0.1650	0.1650	0.0385	0.0385	+ 7	+27	Blood pressure: 135 mm. Hg. throughout experiment
	Vagus stimulation	0.1847	0.1430	0.1320	0.0417	0.0495			
6 April 17	Normal	0.2035	0.1595	0.1650	0.044	0.0385	+25	+13	Blood pressure: 85 mm. at beginning and 75 mm. Hg. at end of experiment.
	Vagus stimulation	0.1650	0.1100	0.1211	0.055	0.0440			
7 May 6	Normal	0.2090	0.1705	0.1540	0.0385	0.0550	±00	−12	Blood pressure: 80 mm.
	Vagus stimulation	0.1705	0.1320	0.1210	0.0385	0.0490			
8 May 21	Normal	0.1420	0.1155	0.0990	0.0165	0.0330	±00	−80	Blood pressure: 80 mm. at beginning of experiment. During the time of taking sample of blood at time of vagus stimulation, the blood pressure rose 10 mm.
	Vagus stimulation	0.1155	0.0990	0.1100	0.0165	0.0050			

CHANGES IN IODINE CONTENT OF THE THYROID GLAND FOLLOWING CHANGES IN THE BLOOD FLOW THROUGH THE GLAND

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This work was undertaken at the suggestion of Dr. Carlson, primarily to determine the influence of changes in the circulation of the thyroid gland on the iodine content of the gland. This point does not seem to have been controlled in the recent interesting work of Rahe, Rogers, Fawcett and Beebe, (1) on the effect of stimulation of the thyroid nerves on the iodine content of the gland. It seems clear that the data reported by Rahe, et al, cannot be construed as proving the presence of secretory nerves to the thyroids, until it is shown that the vaso-motor changes in the thyroid induced by stimulation of the thyroid nerves are not by themselves capable of inducing the changes in the iodine content.

II. LITERATURE

According to Rhinehart (2) the nerve supply to the thyroid is made up of filaments from the cervical sympathetic, entering the gland with the perivascular connective tissue and tunica adventitia of the thyroid arteries. In man and the cat the filaments have been demonstrated to arise only from the superior cervical ganglion of the sympathetic. In the dog some observers claim that nerve filaments from the vagus also enter the thyroids. This is denied by Rhinehart. In the thyroid glands the nerves form elaborate perivascular nerve plexuses and end, at least in part, in the walls of the blood vessels.

Ossakin (3) gives a brief review of the literature on the vaso-motor and possible secretory function of the thyroid nerves. Brian stimulated the vago-sympathetic and inferior laryngeal nerves and studied the histological and vaso-motor changes produced in the thyroids. He concluded that these nerves carry both secretory and vaso-motor fibers. The vaso-motor function has been described as the sole function of the

thyroid nerves by Sacerdatti. V. Cyon reports experiments showing on stimulation of the nervus thyroideus superior, a slight diminution in the carotid blood pressure with a change in the volume of the gland. By measuring the blood pressure in the arteria thyroidea superior V. Cyon concluded that both vaso-constrictors and vaso-dilators are contained in the laryngeal nerves, the constrictors predominating in the superior.

Morat and Sinakewitsch by plethysmography showed that stimulation of the cervical sympathetic gives a pressor effect—a decrease of the amount of blood in the thyroid gland. The latter investigator showed that on extirpation of the superior cervical ganglion or paralysis of this ganglion with nicotine no effect on the thyroid was obtained on stimulation of the sympathetic. From this he concluded that the sympathetic fibers are synapsed in the superior cervical ganglion before reaching the thyroid gland.

Katzenstein reports marked degenerative changes in the thyroid on cutting the thyroid nerves, and concludes that the nerves contain true sensory fibers. These degenerative changes on cutting the nerves are attributed solely to changes in the blood supply by Luebeke.

Wyss and Andersson failed to obtain histological change in the thyroid following injection of pilocarpine.

Huerthle stained the thyroid gland after stimulation of the layrngeal nerves and found no change. He concludes, therefore, that these nerves are not secretory but vaso-motor.

Martini by stimulating and Horsley by cutting the thyroid nerves report no change in the histology of the gland.

Wiener cut the vago-sympathetic and paralyzed the superior cervical ganglion reporting no histological change in the thyreoglobulin content of the gland. But when the inferior cervical ganglion was removed leaving the superior intact he reports a decrease in the weight of the gland and decrease in the thyreoglobulin content, from which he concludes that the thyroid nerves have both a trophic and secretory function.

Asher and Flack (4) working on rabbits, cats and dogs compared excitability of the depressor nerve before and after stimulation of the thyroid nerves, and also the effect of a small intravenous injection of adrenalin before and after such stimulation. They conclude that under exactly similar experimental conditions the excitability of the depressor nerve is increased during or immediately following the stimulation of the thyroid nerves. They also report that this stimulation of the thyroid nerves sensitizes the animal to epinephrin. They as-

cribe these effects to the action of an increased quantity of thyroid secretion poured into the blood on stimulation of the thyroid nerves. Stimulation of the thyroid nerves failed to produce these effects after the extirpation of the thyroid gland. They also claim that intravenous injections of thyroid extract act similarly to stimulation of the thyroid nerves.

The work of Asher and Flack was repeated and apparently confirmed by Ossakin (3) working in Asher's laboratory.

According to the most recent work of Rahe and co-workers (1) stimulation of the nerves to one thyroid lobe decreases its iodine content as compared with that of the control lobe. They conclude that the physiologically active substance of the thyroid is discharged into the circulation on stimulation of the thyroid secretory nerves.

III. EXPERIMENTAL METHODS

All of the experimental work was done on dogs.

Analyses were made on the glands of ten normal animals to determine the relation of the iodine content in the two lobes. The glands were analyzed in duplicate, for iodine content according to Hunter's method (5), modified according to Koch (6). Part of each lobe was dried to constant weight for total solids.

Two series of stimulation experiments were run to determine whether the stimulation of the nerves leading to one lobe causes a diminution in its iodine content. The stimulation was applied to the cervical sympathetic, isolated from the vagus sheath, and to the nerve filaments accompanying the superior thyroid vessels.

The control and stimulated lobes were, in a number of cases, stained for the active secretion to determine whether the stimulation produced histological changes parallel with the chemical changes.

The vascular changes produced by stimulation of cervical sympathetic and the thyroid nerves, were studied both by the rate of the venous outflow and by the plethysmographic methods.

A series of ten animals was run in which the blood supply to one lobe was mechanically interfered with to an extent comparable to that produced on stimulation of the nerves. The main thyroid artery was cleaned of all connective tissue and accompanying nerve filaments. The blood supply was momentarily, (1–2 seconds), cut off, by compressing the artery for two seconds at ten second intervals.

Dogs with slightly enlarged thyroids were selected provided the two lobes were very nearly the same size. Having the pick of a large amount of material it was not difficult to select dogs of this type. Glands of this type were chosen in order to have a sufficient amount of material to run duplicate iodine determinations and to dry a portion for total solids.

The details of the analytical procedure are as follows: The fresh glands were carefully trimmed of all connective tissue and blood vessels and accurately weighed; they were then hashed in a lead-pan, re-weighed in the pan from which a portion was weighed into a carefully weighed glass dish to be dried for total solids, and the two other portons weighed into nickel crucibles and covered with 6 grams of pulvierized sodium hydrate and 2.6 grams of postassium nitrate. The crucibles were then placed on the steam bath for a few hours, the material going into solution in its own liquid. The crucibles were placed in the drying oven and heat applied slowly at 110°C. running up to 140° to 150°C. A little experience made it possible to remove the material at a stage at which it was easily pulverized. It was then mixed with 6.4 grams of fusion mixture and covered with 10 grams of the same, according to Hunter.. From this point on the method as modified by Dr. Koch (6) was followed exactly. Care was taken to dry the material to constant weight in order to determine whether the diminution in iodine on stimulation calculated on the basis of fresh tissue is also true when calculated on the basis of dried substances.

In the stimulation experiments both lobes were left *in situ* until the end of the period of stimulation in every case. We cannot consider a lobe removed before three hours of anesthesia a control of the one removed at the end of this period since we have no knowledge of the nature or extent of the changes which may take place during this time. Light ether anesthesia was used in every case. The nerves were stimulated by brief periods of weak tetanizing current. The effectiveness of the stimulus was controlled by the effect on the pupil, the current used being 3–4 times stronger than the minimum for pupil dilatation. Gruber (7) has shown that the threshold stimulus for the vaso-constriction function of the cervical sympathetic is 6.95 B units while that for the pupil dilation is only 2.27 B units.

Dr. R. R. Bensley has devised a new stain for the thyroid secretion. Portions of several of the stimulated and control lobes were prepared in Dr. Bensley's laboratory and stained by him with a special stain for the thyroid secretion.

IV. RESULTS

Our results in the control series of normal dogs are summarized in Table I. It is evident from this table that the iodine content per gram varies widely in different individuals, but *is practically identical in the two thyroid lobes of the same animal.* The average difference between the two lobes in the case of the fresh tissue was 0.00157 per cent; in case of the dried tissue 0.01488 per cent.

According to Marine (8) the iodine content per gram is practically identical in the two thyroid lobes in dogs.

It is, therefore, evident that the per cent of iodine in one lobe of the thyroid will serve as control of the other, provided one is not dealing with diseased glands.

In the first series of stimulation experiments the cervical sympathetic was stimulated after isolation from the vagus sheath. The data are given in Table II. The average loss in per cent fresh tissue, of iodine in the stimulated lobe was 0.00794 but in Dog I, in which the per cent of iodine was highest, the loss on stimulation was 0.01499 per cent per gram of thyroid or approximately 12 per cent iodine. Dogs I–V were fed about 25 grains of potassium iodide per day for three days previous to the experiment. This was done to insure the presence of iodine in the gland and was discontinued after coming to the conclusion that with the method of Hunter iodine could be detected in every gland even though we were dealing in many cases with hyperplasia. It is interesting to note that in Dog I where the loss was greatest the stimulation period was $3\frac{3}{4}$ hours; in Dog XII where the loss was least the stimulation lasted only $1\frac{1}{2}$ hours. Dog XI did not give a decrease on stimulation, the probable explanation being that we stimulated the lobe with the greater per cent of iodine and the loss was not enough to make it less but only tending to equalize the amounts. On the whole our results show the greater decrease in the iodine content the longer the period of stimulation of the thyroid nerves.

In the second series the thyroid nerves were stimulated near their entrance into the gland with the superior thyroid artery. The blood supply was not mechanically interfered with. The results of this series are given in Table III. It will be seen that the stimulation caused a decrease in the iodine content in every case except Dog II. The average iodine loss per fresh tissue, excepting Dog II is 0.00510 per cent. The average loss calculated for dry tissue is 0.03516 per cent.

The results of these two series confirm those of Rahe, et al, but the

percentage loss is not as great as reported by them. It is apparent from Tables II and III that there is in nearly every case a greater per cent of total solids in the case of the stimulated lobe. Of course the per cent of total solids varies somewhat in the two lobes of the same animal in the normal series, (Table I), but it is obvious that we could not have chanced in every case, to stimulate the lobe with the smaller water content.

As stated before, the vascular changes, produced in the thyroid on stimulation of the thyroid nerves, were studied by the rate of venous

Fig. 1. Showing vaso-constriction in the thyroid gland on stimulation of the cervical sympathetic nerve. *A*, carotid (left) blood pressure. *B*, pehethysmograph record of the right thyroid lobe. *C*, showing stimulation of right cervical sympathetic nerve. *D*, time 3½ seconds.

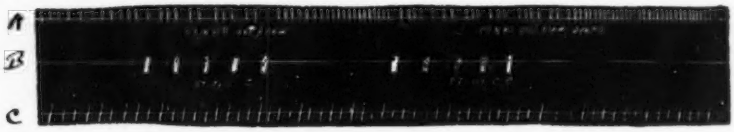

Fig. 2. Retardation and subsequent acceleration of the venous blood flow from the thyroid vein on momentary stimulation of the cervical sympathetic nerve. *A*, record of drops of venous flow. *B*, stimulation of cervical sympathetic nerve. *C*, time 3½ seconds.

outflow and by the plethysmograph. The cervical sympathetic was ligated and stimulated centrally with the same strength of current as in our stimulation experiments with the iodine determination. The results with the first method are summarized in Table IV (see also fig. 2). Even a stimulation lasting only 1–2 seconds cause a distict diminution in the venous outflow (exp. 8, 9). In some cases the vaso-constriction during the stimulation is followed by vaso-dilation on cessation of stimulation (fig. 2). The plethysmograph shows marked decrease in the volume of the gland on stimulation of the cervical sympathetic or the thyroid nerves (fig. 1) . Our experiments then confirm the work

of Ossakin (3). The cervical sympathetic supplies vaso-constrictor fibers to the thyroid.

A series of ten experiments were run in which the temporary and periodic decrease in the blood flow through the thyroid gland was produced mechanically and without any stimulation of the thyroid nerves as previously described. The iodine and total solid determination of the thyroids in this series are given in Table V. In every case there is a diminution in the percent of iodine in the lobe in which the blood flow had been periodically decreased. The decrease in iodine is not as great as in the series of stimulation of the thyroid nerves (Tables II and III). Control experiments showed that by one method of periodic compression of the superior thyroid artery there was less diminution of the blood flow through the gland than on stimulation of the thyroid or the cervical sympathetic nerves. In 80 per cent of the cases in this series the per cent of total solids is greater in case of the lobe whose blood flow was periodically diminished. It is clear that temporary vaso-constriction of the thyroid vessels, or temporary decrease in the blood flow through the thyroids induced by momentary compression of the thyroid arteries and without stimulation of the thyroid nerves produce the same changes in the thyroid gland as does the stimulation of the thyroid nerves, that is, a decrease in the iodine and water content. Since the variations in the blood flow is by itself capable of inducing these changes, and since these vascular changes are produced in the gland on the stimulation of the thyroid nerves, it is obvious that these effects of the nerve stimulation do not prove the presence of true secretory nerve fibers to the thyroid glands.

Dr. Bensley's special stain showed no difference between the control and stimulated lobes of the same gland.

TABLE I

Control series. (Dog)

Iodine content of right and left thyroid lobe of same animal

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH GLAND	% OF TOTAL SOLIDS	% OF I IN FRESH TISSUE	% OF I IN DRIED TISSUE	AV. DIFF. IN % OF I IN 2 LOBES FRESH TISSUE	AV. DIFF. IN % OF I IN 2 LOBES DRY TISSUE
I	R	38.2	20.5	0.03432	0.16335	0.00346	0.01686
	L	36.5	20.8	0.03778	0.18021		
II	R	26.0	19.5	0.01737	0.08914	0.00153	0.00850
	L	19.1	18.5	0.01584	0.08564		
III	R	14.5	24.2	0.30336	0.13787	0.00212	0.00982
	L	15.1	24.4	0.31024	0.12805		
IV	R	10.7	27.6	0.08886	0.32197	0.00398	0.06787
	L	10.1	33.8	0.08588	0.25410		
V	R	10.5	30.6	0.04172	0.13625	0.00172	0.02767
	L	6.6	26.5	0.04344	0.16392		
VI	R	19.2	25.0	0.00636	0.02548	0.00031	0.00078
	L	18.9	27.2	0.00667	0.02470		
VII	R	23.0	20.4	0.00732	0.03593	0.00019	0.00352
	L	18.6	22.0	0.00714	0.03341		
VIII	R	9.2	20.4	0.00666	0.03265	0.00108	0.00605
	L	10.2	20.4	0.00774	0.03870		
IX	R	53.6	20.3	0.00762	0.03754	0.00016	0.00085
	L	41.8	20.3	0.00778	0.03839		
X	R	49.7	22.0	0.01284	0.05838	0.00111	0.00744
	L	60.0	21.2	0.01395	0.06582		
					Average differences:	0.00157	0.01488

TABLE II

(*Dog*)

Effect on iodine content of thyroid of stimulation of the cervical sympathetic nerve

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH LOBE	% OF TOTAL SOLIDS	(A) % OF I IN FRESH TISSUE	(A) % OF I IN DRIED TISSUE	LOSS IN % FRESH TISSUE	LOSS IN % DRIED TISSUE	STIMULATION TIME
I	L. stim.	14.4	29.2	0.12776	0.43715	0.01499	0.04640	3 hrs. 45 min.
	R. cont.	14.2	29.5	0.14265	0.48355			Int. 10 sec.
II	L. stim.	9.4	23.8	0.03609	0.15167	0.00717	0.05800	3 hrs.
	R. cont.	8.3	21.0	0.04326	0.20967			Int. 10 sec.
III	R. stim.	13.2	25.4	0.06072	0.23905	0.01364	0.10603	4 hrs.
	L. cont.	14.2	23.0	0.07436	0.34508			Int. 10 sec.
IV	R. stim.	12.5	23.1	0.04034	0.17469	0.01179	0.07356	3 hrs.
	L. cont.	13.9	21.0	0.05213	0.24825			Int. 10 sec.
V	R. stim.	19.6	30.1	0.05498	0.18267	0.00502	0.04037	2 hrs.
	L. cont.	20.4	26.9	0.06000	0.22304			Int. 3½ sec.
VI	R. stim.	15.7	22.3	0.01664	0.07462	0.00225	0.01895	3 hrs., 30min.
	L. cont.	16.5	20.2	0.01889	0.09357			Int. 8 sec.
VII	L. stim.	31.0	23.2	0.03175	0.13680	0.00612	0.03459	3 hrs.
	R. cont.	31.4	22.1	0.03787	0.17139			Int. 10 sec.
VIII	R. stim.	38.3	28.7	0.03746	0.13052	0.00461	0.00607	3 hrs., 15 min.
	L. cont.	31.8	30.8	0.04207	0.13659			Int. 10 sec.
IX	L. stim.	10.8	24.3	0.02693	0.11087	0.00418	0.04153	3 hrs.
	R. cont.	8.1	23.7	0.03111	0.15240			Int. 10 sec.
X	L. stim.	11.9	24.4	0.01667	0.06861	0.00966	0.03974	3 hrs., 45 min.
	R. cont.	11.7	24.3	0.02633	0.10835			Int. 10 sec.
XI	L. stim.	18.1	21.0	0.01452	0.06914	0.00167	0.00795	2 hrs., 30 min.
	R. cont.	19.2	21.0	0.01285	0.06119			Int. 10 sec.
XII	R. stim.	21.0	20.9	0.00103	0.00500	0.00035	0.00166	1 hr., 30 min.
	L. cont.	22.5	20.7	0.00138	0.00666			Int. 10 sec.
				Average loss in per cent		0.00794	0.04572	

(A) Average of duplicate analyses.

TABLE III

(*Dog*)

Effect on iodine content of thyroid of stimulation of the thyroid nerves near the entrance into the gland.

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH LOBE	% OF TOTAL SOLIDS	% OF IODINE FRESH TISSUE	% OF IODINE DRIED TISSUE	% OF LOSS OF IODINE FRESH TISSUE	% OF LOSS OF IODINE IN DRIED TISSUE	TIME OF STIMULATION
I	R. stim.	12.6	23.8	0.01299	0.05450	0.00152	0.07870	3 hrs., 45 min.
	L. cont.	8.3	18.4	0.02451	0.13320			Int. 10 sec.
II	R. stim.	15.1	24.3	0.01491	0.06135	0.00238	0.01260	3 hrs.
	L. cont.	14.3	25.7	0.01253	0.04875			Int. 10 sec.
III	R. stim.	5.6	27.0	0.03883	0.14380	0.00463	0.02866	3 hrs.
	L. cont.	6.8	25.2	0.04346	0.17246			Int. 10 sec.
IV	L. stim.	3.9	22.8	0.03480	0.15263	0.00639	0.05229	3 hrs., 15 min.
	R. cont.	3.9	20.1	0.04119	0.20492			Int. 10 sec.
V	R. stim.	15.4	22.5	0.00343	0.01524	0.00103	0.00589	2 hrs., 45 min.
	L. cont.	16.8	21.1	0.00446	0.02113			Int. 3½ sec.
VI	L. stim.	12.7	21.8	0.00223	0.01022	0.00193	0.01027	3 hrs.
	R. cont.	12.7	20.3	0.00416	0.02049			Int. 10 sec.
	Average loss except No. II					0.00510	0.03516	

TABLE IV

The influence of stimulation of the cervical sympathetic and thyroid nerves on the blood flow in the thyroid gland. In Exp. 1-3 the figures are given in cc. of blood per minute; in Exp. 4-9 the figures are given in number of drops of blood per 20 seconds. In Exp. 8 and 9 the stimulation lasted only 2 seconds.

EXPERIMENT NO.	BEFORE STIMULATION	DURING STIMULATION	AFTER STIMULATION
1	17.5	13	16
2	15.0	12	13
3	9.0	6	8
4	38.	28	36
5	36.	25	33
6	88	22	28
7	38	23	31
8	40	28	32 (2)
9	24	16 (1)	24 (2)

(1) 5 seconds after stimulation (2) 10 seconds after stimulation

TABLE V

The effect on the iodine content of the thyroid gland of periodic diminution of the blood flow through the gland, occlusion of the main thyroid artery for 1–2 seconds every 10 seconds.

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH LOBE	% OF TOTAL SOLIDS	% OF I IN FRESH TISSUE	% OF I IN DRIED TISSUE	DIFF. IN % OF LOSS IN FRESH TISSUE	DIFF. IN % OF LOSS IN DRIED TISSUE	TIME OF INTERF. CIRC.
I	Right	38.5	24.4	0.00335	0.01372	0.00272	0.01094	3 hrs., 15 min.
	L. cont.	37.9	24.2	0.00607	0.02466			
II	Left	40.2	21.1	0.00166	0.00786	0.00126	0.00719	3 hrs.
	R. cont.	28.9	19.4	0.00292	0.01505			
III	Left	3.9	20.8	0.01324	0.06365	0.00229	0.00694	3 hrs., 30 min.
	R. cont.	3.0	22.0	0.01553	0.07059			
IV	Left	14.0	25.1	0.00960	0.03824	0.00220	0.00896	3 hrs.
	R. cont.	9.5	25.0	0.01180	0.04720			
V	Right	6.5	21.6	0.00432	0.02000	0.00148	0.00723	3 hrs.
	L. cont.	11.7	21.3	0.00580	0.02723			
VI	Left	8.3	23.1	0.04281	0.18530	0.00532	0.04389	3 hrs.
	R. cont	8.0	21.0	0.04813	0.22919			
VII	Left	10.8	22.3	0.03256	0.15964	0.00422	0.02243	3 hrs.
	R. cont.	10.2	20.2	0.03678	0.18207			
VIII	Right	14.5	24.3	0.02613	0.10753	0.00671	0.03061	3 hrs., 15 min.
	L. cont.	15.8	23.7	0.03284	0.13814			
IX	Right	32.0	21.0	0.05548	0.26419	0.00111	0.00528	3 hrs.
	L. cont.	29.2	21.0	0.05659	0.26947			
X	Right	3.9	22.0	0.01280	0.05818	0.00290	0.01587	2 hrs., 40 min.
	L. cont.	3.4	21.2	0.01570	0.07405			
				Average		0.00312	0.01593	

SUMMARY

1. The iodine percentage of the thyroid lobes in dogs is practically identical (Table I).

2. Stimulation of the cervical sympathetic nerve or the thyroid nerves induces a decrease in iodine and water content of the thyroid glands (Tables II and III).

3. The stimulation of the cervical sympathetic or the thyroid nerves induces vaso-constriction in the thyroid gland (Table IV).

4. Decrease in the thyroid blood flow similar to that induced by stimulation of the above nerves, but produced by mechanical means, causes a decrease in iodine and water content of the thyroid gland (Table V).

5. The changes in the thyroids so far shown to be caused by the stimulation of the cervical sympathetic or the thyroid nerves can all be accounted for by the vascular changes.

6. No histological changes could be demonstrated following stimulation of the thyroid nerves or mechanical interference with the blood flow.

I wish to express my gratefulness to Dr. Carlson for his supervision; to Dr. Koch and Mr. Maurer for their valuable suggestions in the analytical work; and to Dr. Bensley for his help in the histological work.

ADDENDUM

These observations appear to place the thyroid gland in the same category as the liver, that is to say, as organs very delicately balanced in relation to the local circulation, irrespective of any secretory nerves. Since the periodic stimulation of the thyroid nerves induce primary vaso-constriction followed by vaso-dilatation, one cannot say at present whether the vaso-constriction or the vaso-dilation is the active factor in bringing about the discharge of iodine from the thyroid gland, or at any rate upsetting the balance between iodine absorption and iodine discharge. The thyroids have a greater blood supply than any other organ in the body. This tremendous blood supply has in all probability a far greater significance than the mere oxygen supply and the carbon dioxide removal.

The fact that relatively transient vaso-motor disturbance in the thyroid induces such marked change in thyroid activity is probably of significance in certain types of goitre in man.

The observations also offered another illustration of the difficulty in deciding whether we are dealing with vaso-motor nerves or with secretory nerves in certain organs. The adrenal glands probably also fall in this group, although most investigators interpret the increased discharge of adrenalin following stimulation of the adrenal nerves as a true secretory nerve action.

A. J. C.

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PHOTOELECTRIC CURRENTS IN THE EYE OF THE FISH[1]

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I. INTRODUCTION

It is a fact well known that electrical phenomena are intimately associated with the normal activity of muscle and nerve. Of later experimental acquisition however is the knowledge that the sense organs fall within the same category; both the eye and the ear are accompanied in their normal functioning by electric currents. A beam

[1] The substance of the present paper was sent to the Arch. f. Anat. u. Physiol., July 15, 1914, under the title "Die photoelectrischen Vorgänge im Auge des Fisches," together with the original photographic records. Since the war has indefinitely delayed the appearance of the paper in that publication, no word having been received regarding it after a year has elapsed from the date of sending, I have deemed it only fair and proper that the results should be published in one of the American journals.

of light falling upon the eye sets up a complex change of electrical potential in the retina, while sound vibrations generate electric currents in the cochlea.

The frog and higher vertebrates have been the animals upon which the photoelectric currents have been most successfully studied. The phenomena which investigators have collectively brought to light by such a study are these:

When a dark-adapted eye is connected with an instantaneously reacting galvanometer, after placing one electrode on the cornea and the other on the back of the eyeball, and a brief exposure is made of the eye to light, the galvanometric deflections are, three (a negative followed by two positive) for the "make" of light, and one (positive) for the "break of light." The usage of the terms positive and negative will presently be explained. All four of these deflections are obtainable for the eye of the frog, turtle, chicken, owl, dove and monkey. Variations occur for eyes of the cat and the dog. The studies of the phenomenon in the eye of the fish have, for reasons to be mentioned, been attended with meagre success.

The galvanometers at first employed were too sluggish to respond at all to the fleeting retinal currents; and even when instruments were found which registered the electric effects for the eye of the frog, the same instruments and methods when applied to the fish were without result. Whereas the extirpated eye of the frog is capable of yielding strong retinal currents long after the operation, the extirpated eye of the fish, on the contrary, loses that ability so rapidly that, by the time the operator is ready to experiment, the currents are too feeble to record. In order to overcome this obstacle one must work with the living fish. The difficulty, however, of keeping the animal's eye slightly above water in order to apply electrodes to it, and the rest of its body below the surface in order that it may breathe, has been perhaps the chief factor which has deflected investigation of the phenomenon in the retina of the fish to a more intensive study of it in the frog and higher vertebrates. It was with the purpose of supplying this lack of data for a purely aquatic animal that the present research was undertaken.

The work was carried on in the Physiological Institute of Berlin University during the winter semester of 1912–1913. To Prof. Max Rubner, director of the Institute, I am greatly indebted for the unstinted privileges of research granted to me. The problem was proposed to me by Professor Piper, and to him for his kind supervision and valuable suggestions throughout the work I desire here to express my

thanks. I wish also to thank Dr. Arnt Kohlrausch for much kind assistance. The opportunity for the sojourn in Berlin was afforded by the grant of a Sheldon Fellowship from Harvard University.

There are certain matters of definition which should be made clear at the outset. Waller (22) found difficulty in reconciling statements of previous observers with reference to the direction of the current at "make" and "break" of light, "because that that direction was so frequently characterized as positive and negative with reference to a 'current of rest' which was itself of variable direction." Since later investigators, in agreement with Waller, have found the current of rest (i.e., the current occasioned simply by placing the cornea in circuit with the back of the eyeball) taking the direction from fundus to cornea within the eye and from cornea to back of eyeball in the outside circuit, this direction has been regarded as positive. Since in the fish eye the current of rest takes the same direction, the term positive will here be used in the same sense. In the photographic curves it is always an upward deflection.

Gotch (9) introduced the terms "On effect" and "Off effect" to designate the initial and terminal effects at make and break of light; but since his "On effect" was primarily attached to the first positive deflection in the photographic curve, I wish to employ the term to cover the negative preliminary depression and the secondary positive rise in the curve as well, unless otherwise qualified. The term "Off effect" will always refer to the simple positive deflection occurring at the break of light. In order to refer to the individual features of the curve more easily, Einthoven and Jolly (8) have been followed in denominating the negative preliminary deflection as A, the first positive elevation as B, and the secondary on-effect as C; but instead of adopting A′ to designate the off-effect (as E and J do) which associates it upon hypothetical grounds with the on-effect A, the non-partisan letter D has been given it.

II. HISTORICAL REVIEW

In the year 1849 du Bois Reymond (7) made the discovery that the retina of the fish reacted electromotorically. An extirpated eye of the tench, with one electrode on the cornea and the other either on the cross-section or on the longitudinal axis of the optic nerve, gave an electric current. An action-current could similarly be obtained by connecting the cross-section of the optic nerve with its long axis. It occurred to him to test further for the effect of light striking the retina.

but his experiment on a turtle with the aid of a multiplicator, an instrument responsive only to the stronger currents generated during muscular contractions, yielded no results.

It remained therefore for the Swede Holmgren (12, 13) to discover the photoelectric reaction for which du Bois Reymond had sought in vain. The electric currents evoked by light which he detected in the eye of the frog and the rabbit, however, he was unable to elicit with surety from the eye of the fish. The experiments were performed upon extirpated eyes of pike, perch and bleak, but the results obtained were so fleeting and irregular that, although on- and off-effects were obtained once or twice, the question remained a matter of uncertainty.

The investigations of Dewar and M'Kendrick (6) on the eyes of the goldfish, rockfish and stickleback with the aid of a Thompson galvanometer met with better success. From the goldfish (Cyprinus auratus) a positive on-effect was obtained and a negative off-effect; but whether the former was the B or C deflection was not distinguished. Similar though slower reactions were obtained for the rockfish (Motella vulgaris). In the case of the stickleback, the authors remark (p. 61): "A deflection of 400° was obtained, which was remarkably sensitive to light, the variations occurring in very short periods of time." The conclusion drawn from the experiment was that the differences in the reaction-times of the various fish was to be attributed to the different habits of the animals; or, in the authors' words, "on comparing these results, it will be seen that in the active alert stickleback, the variations occurred rapidly; in the more sedate goldfish, they occurred more slowly, while, in the case of the sluggish rockling, which hides beneath stones near low-water mark, the variations occurred very slowly."

Kühne and Steiner (16), in working with enucleated eyeballs of perch, pike, barbel and eel, obtained the positive on-effect as had already been observed by Dewar and M'Kendrick, but instead of a negative deflection at break of light, they got a feeble positive effect. When cornea, iris and lens were cut away, the posterior half of the eyeball yielded the same result, only that in this case the off-effect was decidedly positive. From the isolated retina too, provided that it was strictly fresh, were obtained similar though feebler results. In all cases the current remained constant during illumination as soon as the first positive deflection had subsided. Again the negative preliminary (A deflection) escaped notice because the instrument used was too slow in reaction to register it. It is also likely that the positive on-effect observed by these investigators was the secondary C crest rather than the more

rapid B deflection, judging from the statement, "Die Schwankungen der Retinaströme sind beim Kommen des Lichtes positiv sehr langsam wachsend, gegen das Ende rasch zum Maximum gehend, während des Belichtens anhaltend."

From 1881 to 1907 attention was concentrated upon the phenomenon as exhibited by the eyes of the frog and higher vertebrates, while study of them on the fish was neglected. The researches of v. Brücke and Garten (2), carried on with a more efficient instrument in the small Einthoven string galvonometer, were extended to cover aquatic as well as terrestrial vertebrates. The fish used were the pike and the bleak. In experiments with the former, where the electrodes were applied to the eye *in situ*, the on-effect obtained was described as "eine positive Schwankung des normal gerichtetet Bestandstromes, die während der Belichtungsdauer an Stärke zunahm," and the off-effect as "eine positive Schwankung, nach der der Strom allmählich zu seinem Ruhewert zurück kehrte." From the enucleated eyeball, however, the on-effect was negative while the off-effect was positive. For the bleak only extirpated eyes were used. The current of rest obtained was strong and ran in the outer circuit at first from the back of the eyeball to the cornea, then, diminishing rapidly to zero, flowed in the reverse or normal direction. These investigators were able to detect a negative preliminary as a feature of the on-effect, the latent period of which was 0.074 sec. in one instance, and 0.075 sec. in another. Following this negative preliminary came a positive deflection with a latent period measuring 0.128 sec. and 0.132 sec. in the two cases respectively. On darkening, the eye of the bleak yielded no definite reaction and the curve subsided gradually to zero. The combined results from both kinds of fish exhibited therefore the full set of phenomena known to occur in the frog retina, viz., the initial negative depression and the two positive deflections as the on-effect, and the positive deflection as the off-effect.

Since the work of v. Brücke and Garten no further attempt had been made to verify or add to these facts regarding the photoelectric phenomenon in the eye of the fish. The substitution of the quick-reacting string galvanometer for the older and more sluggish instruments, enabled Einthoven and Jolly (8) to analyse the currents in the eye of the frog far more carefully than had been done before, and Piper (20) to make a thorough comparative study of the photoelectric effects in the eyes of the turtle, dove, chicken, buzzard, owl, rabbit, cat, dog, monkey and octopus as well as the frog. Since the fish was not included

in this set of investigations, it was with the object of supplementing the series in that direction that, first with the aid of a d'Arsonal galvanometer and finally with a large Einthoven string galvanometer I carried out the following experiments.

III. EXPERIMENTS AND OBSERVATIONS

A. With d'Arsonval galvanometer

The kinds of fish which were used in the experiments were the tench (Tinca tinca L.) and the pike (Esox lucius L.) These are freshwater forms common to northern Germany and were obtainable throughout the winter months of 1912–13 during which the investigation was carried on. The pike, although a less hardy fish than the tench, yet, because of its larger eyes and more convenient shape, proved the more satisfactory; it alone therefore served as material throughout the chief part of the work. The fish were obtained fresh from the market every two or three days as needed and were kept in a laboratory aquarium supplied with running water.

The d'Arsonval galvanometer was employed in the earlier experiments, but as soon as the technic of working with the live animals was mastered, resort was made to the Einthoven string galvanometer in conjunction with the Hut photographic register for making the final records.

The experiments with the d'Arsonval instrument were performed on two tench and four pike. In order that the electrodes might be placed upon the eye in a dry condition, the fish was wrapped first in a wet cloth and then in a wire net to prevent any bodily movement. Thus encaged the animal was laid on its side upon bags of sand in a trough of water so as to immerse the snout and as much of the rest of the body as possible, one eye being kept above the surface. While in this position the fish could without difficulty take in water through the mouth and irrigate the gills, and it seemed to suffer no inconvenience during the hour or two of experimentation. The water was aerated with compressed air. The trough was placed in a large black box with black curtains in front. Light from a 32 C. P. electric light, set at about a meter distant, passed through an aperture in the rear of the box and was reflected from a mirror situated inside onto the eye of the fish. Before being placed in position in this dark chamber the eyeball was partially laid bare by a slight operation so that one elec-

trode might be brought in contact with the eyeball and the other with the cornea. The meshes of the wire net enveloping the fish were widened over the eye sufficiently to admit the electrodes, contact from which being made by means of short woolen threads saturated with physiological salt solution. The temperature of the water did not vary more than a degree or two centigrade from that of the room (av. 17°C.). For the first five experiments the animal was not dark-adapted; but thereafter it was placed, after the operation on the eye had been performed, in a light-proof tank of running tap-water for about three hours previous to the experiment.

TABLE I.

Photoelectric currents in the eyes of the tench (T) and pike (P) as measured by a d'Arsonval galvanometer.

EXPERIMENT	DATE 1912	FISH	NO. HOURS DARK-ADAPTATION	DEFLECTION ON GALVANOMETER		
				Current of rest	On effect	Off effect
1	XI. 12	T. 3	0	+6. cm.	+0.5 cm.	0 cm.
2	XI. 12	T. 3	0	+4.	+2.	0
3	XI. 15	T. 3	0	+12.	+0.2	0
4	XI. 15	T. 4	0	− 3.	+ 1.	0
5	XI. 17	P. 1	0	+20.	+0.5	+0.1
6	XI. 17	P. 1	3	+25.	+11.	0
7	XI. 19	P. 1	?	−25.	+0.2	0
8	XI. 22	P. 2	3	−4. 8	−0.8	0
9	XI. 25	T. 4	1.5	+15.	+2.	0
10	XI. 26	P. 3	3	+11.	+ 6.	0
11	XII. 3	P. 4	3	?	+ several cm.	?

The results of observations made with the aid of the d'Arsonval galvanometer are given in Table I. Column three indicates the kind of fish used (T. for tench, P. for pike) and the animal's number; column four the period of adaptation to dark; columns five, six and seven give the throw in centimeters on the galvanometric scale for the current of rest[2] and for the on- and off-effects of light respectively. The plus sign signifies a current flowing in the outer circuit from cornea to back of eyeball, and the negative sign the reverse direction. It will be seen that in seven of the eleven cases the current of rest was posi-

[2] Before the beginning of each exposure this current of rest was neutralized by means of an oppositely directed galvanic current.

tive. The last, though unrecorded as to direction, was probably positive too, making a total of eight. Only once (Experiment 8) did the flashing on of the light generate a current whose direction was opposed to that of the normal current of rest, i.e., from back of eyeball to cornea in the outside circuit. In this instance both current of rest and photoelectric current were negative, accounted for no doubt by the feeble condition of the animal, for it died before the end of the experiment.

The results in general showed that the eyes of both tench and pike responded with postive currents to the onset of light and that this effect was enhanced by dark adaptation. A negative preliminary to the positive on-effect, could not be ascertained with this instrument. As for an off-effect, only once or twice could a feeble positive current be observed.

B. With Einthoven string galvanometer

The study of the photoelectric phenomena with the aid of the Einthoven string galvanometer was conducted along two lines: first, the simple effects of making and breaking the light were investigated, and then the compound effect obtained by intermittent stimulation. In the first part of the work both dark-adapted and light-adapted animals were used, but for the experiments with intermittent light only dark-adapted ones were employed. Care was taken to select from the market fish whose physiological condition was sound. This was especially important because the pike, the only fish used in this set of experiments, is not a very hardy animal, and unless procured when in prime condition yields electric currents too feeble to be photographed satisfactorily.

The procedure was modified in some respects from that which was followed in the first series of experiments. The operation was the same: a small part of the orbit overshelving the eye was cut away and the exposed muscles freed from their insertions on the eyeball so that one electrode could be brought into contact with the equatorial region. In some cases the operation was performed in the late afternoon and the fish kept in the dark over night.

An arrangement for holding the fish in an immovable position was devised as follows (fig. 1): a board of sufficient length and width to accommodate the largest animal was provided with a large hole near the end, while many smaller holes were scattered irregularly over its length. The fish was wrapped in a wet cloth, the head and gills alone

being left exposed, and then bound on its side to the board by means of wires inserted in the small holes. The under eye and gills, falling in the open space of the large aperture, were quite free from pressure, and the gills thus had freedom of action when running tap-water was led into the mouth through a rubber hose. The fish had less chance to move in this position than when enclosed in the wire net, and consequently it became somewhat numb from the confinement; but it recovered its muscular activity as soon as it was released and returned to the aquarium. When firmly fastened to the board the fish was laid in a large tray which had an outlet tube in the bottom to drain off the water issuing from the gills. This tray was situated in the large dark-box above mentioned through whose aperture at the back the light from a single Nernst glower was reflected by a mirror onto the eye of the fish. One of the electrodes was applied indirectly to the bulb of the eye by means of a wool thread, while the well moistened clay boot of the other impinged directly on the cornea.

In order to obtain a record on the photographic paper of the moment when the light stimulus was applied, the same apparatus was used which Piper (20) had devised for his experiments on the eyes of higher vertebrates. It consisted of a T-tube (Fig. 1, *t*) containing two mirrors located in the center of the cross-arm and at right angles to each other, so that the light entering the vertical arm was reflected, half of it through one end of the cross-arm into the photographic register (*r*), and the other half through the other end onto the eye. A photographic shutter (*s*) on the end of the vertical arm made time exposures of varying lengths possible. When the shutter was opened both the eye and the photographic paper received a portion of the light simultaneously. A time-marker (*z*), making beats at the rate of five per second, was placed in front of the photographic register so that the shadow of its needle together with the shadow of the string of the galvanometer (*g*) fell upon the sensitized paper. Every photograph thus contained a three-fold record: (1) that of the begininng, duration and end of stimulation; (2) that of the retinal currents produced; (3) a time-scale in fifths of seconds.

1. Single exposures to light

(a) *Dark-adapted Eye.* In the preliminary experiments the degree of dark adaptation of the eye seemed to affect materially the size of the deflections of the galvanometric needle. In this set of experiments therefore attention was paid at first only to the phenomena exhibited

by eyes which had been adapted to the dark for at least three hours. The fixing of the fish to the board, the regulation of the artificial respiration and the placing of the electrodes on the eye, were all done by the light of a red electric globe. Since in the first experiments the current of rest in normal animals was found to flow from cornea to eyeball in the outside circuit, and the on-effect, B, to express itself by a current flowing in the same direction, it was assumed in these experiments that when the B deflection agreed in direction with that of the current of rest,

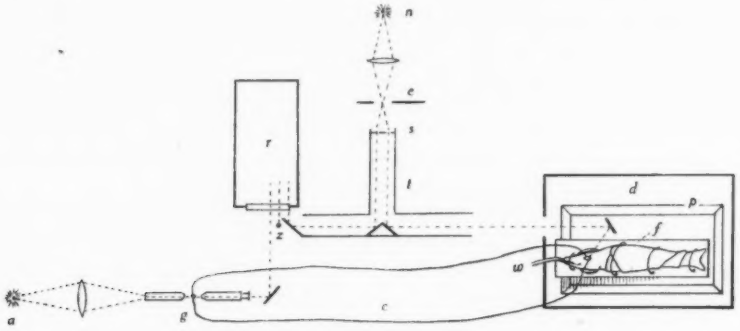

Fig. 1

EXPLANATION OF FIGURE 1

a, arc light
c, electric circuit from eye to galvanometer
d, dark box
e, episcotister
f, fish board
g, string galvanometer
n, Nernst light
p, shallow tray
r, photographic recording apparatus
s, photographic shutter
t, T-tube for dividing the light from *n*
z, time-marker

this direction was from cornea to eyeball in the outside circuit. When the direction was in disagreement, but the on- and off-effects were normal, it was assumed that a polarization current was masking the current of rest. Before the usual neutralization of the current of rest was made, the polarization current was reversed by readjusting the electrodes and brought to concide with it. Instances of this kind however were rare. Be it understood then, that an upward deflection in the curves signifies a current flowing in the outside circuit from the cornea to the back of the eyeball.

It may be stated at the outset that the photoelectric phenomena in the eye of the fish show striking agreement with those exhibited by the frog. The on-effect comprises three features: a negative preliminary A, followed by a quick positive elevation B, and a slower secondary rise C. The off-effect consists of a positive deflection D. These phenomena will be considered first for the dark eye and then for the light eye.

The negative preliminary A appears at some stage or other in each of my eighteen series of records, although not in every individual photograph of a given series. Its absence from some of the photographs will be accounted for later. Suffice it to say at this point that it is typically present for the dark eye. It is preceded by a latent period of 0.032 seconds after the make of light, this value being an average of twenty-nine measurements obtained from the records of five different animals.

On-effect. The positive on-effect B following the negative preliminary never fails to appear except in the record of an animal whose physiological condition was at a low ebb. The latent period for B, measured from the beginning of the light stimulus to the maximum point of the negative preliminary depression, averages 0.075 seconds from thirty-five measurements. The maximum point in the deflection is arrived at on an average in 0.26 seconds. Its amplitude varied considerably in the course of the experiments owing to variations in the resistance of the electrode connections and to differences in the physiological state of the animals. The usual amplitude is about equal to that obtained from a galvanic current of one-tenth millivolt. The maximum is half again as great as this standard amplitude. In the dark-eye this positive on-effect is characteristically large. The ascending leg of the curve is always abrupt while the descending one is quite gradual. In the descending phase the curve sometimes passes below the level of the current of rest and becomes negative. The amount by which it approaches or passes under the zero line is determined largely by the strength with which the slow-developing secondary positive elevation C makes itself manifest.

In animals left in the dark over night and used on the following morning direct from their long period of dark adaptation, this secondary rise following the first positive effect is quite pronounced. With each exposure to light however, it develops at an ever retarding rate and ever diminishing amplitude. It soon disappears altogether and the whole curve following upon B subsides to a constant level which may be even

below that of the current of rest. Since in most instances the C crest developed so slowly that much photographic paper must needs have been consumed in order to obtain its full course, only a single record was made in which its full course was followed. It was a case in which the fish had been dark-adapted over night and the C effect was strong. The period from onset of light to the maximum point in the curve was here seven seconds. The descent was slower than the ascent, and after nearly twenty seconds of its downward course, at which point the record was ended, the curve had not yet returned to the zero line. The duration of exposure in this instance was two and three-fifths seconds. The condition necessary to the production of the secondary elevation seems to be a long interval of dark adaptation.

Off-effect. The positive elevation in the curve, occasioned by the shutting off of the light, has an average latent period of 0.049 seconds. The off-effect increases in magnitude the longer the eye is previously exposed to light. The ascending slope of the D crest is somewhat steeper than the descending. The latter, before reaching the level held by the curve just prior to the break of light, is often arrested in its rapid descent to continue at a more gradual rate downwards. It is quite possible that this second phase in the descent is but the underlying C elevation, upon which the off-effect had been superposed, again cropping out, or it might be due to the still lingering descent of the B crest.

(b) *Light-adapted eye.* Time did not permit an investigation of the photoelectric phenomena in the light eye to an extensive degree. The results obtained, however, are significant enough to be described. In three instances a fish was brought direct from the market, the operation performed in a large porcelain sink by the light of a 50 c.p. electric light, the fish placed in position and a photographic record made. In all three cases the first exposure revealed the fact that the off-effect was decidedly stronger than the on-effect. In one experiment an accident to the apparatus prevented the making of a photographic record, but this decided difference between the on- and off-effects was distinctly observed when the image of the string of the galvanometer was projected onto a screen. In the other two experiments reliable photographic records were secured which fully confirmed this observation. In Plate I are reproduced one set of these records.

Figures 1, 2 and 3 are curves obtained from a single animal during one experiment, showing first a typical light condition (Fig. 1), then a rapid transition to a condition of dark adaptation (Figs. 2 and 3),

in which the comparative magnitude relations of on- and off-effect have become reversed. Examined in detail the curves exhibit these features: Figure 1 (a record made immediately after bringing the fish from the market and without previous dark adaptation), the absence of both A and C deflections, the presence of a diminutive B crest and a strong D deflection; Figure 2 (a record made after ten minutes' dark adaptation following upon Fig. 1), the feeble presence of A, with B strongly developed and D much reduced—the slight elevation following B is not a C crest, as it was occasioned by the movement of the animal's body; Figure 3 (following Fig. 2 after six minutes more dark adaptation), the indisputable presence of the negative preliminary A, a further slight increase in the magnitude of B and slight decrease in the size of D. This last might be attributed to the slightly shorter period of illumination preceding it as compared with the length of exposure in Fig. 1. Thus the typical condition for the light-adapted eye is seen only in the first exposure, and it passes quickly over into that of the dark eye with a comparatively short period of dark adaptation, ten to twenty minutes.

There is another phenomenon in connection with the transition from the light to the dark condition and the reverse to which I desire to call attention. If an eye of intermediate dark adaptation be subjected to light for ten minutes, there is produced at close of exposure a gradual secondary positive rise following the D deflection which may be designated with the letter E. If during this secondary elevation an exposure of about one-eighth second be made, the A, B and D fluctuations appear in the curve without altering its general upward trend. In similar manner, if an eye of intermediate adaptation be subjected to dark for ten minutes and then the brief exposure be made, a strong downward course follows the first positive on-effect B, and upon this descending phase the D crest may be superposed as an off-effect without altering the general downward trend. The off-effect has also been obtained superposed upon the ascending phase of the C crest. Thus the D deflection may crop out upon either ascending or descending phases of other fluctuations such as the C and E crests, or upon the descending phase of the B deflection. But it is also true for the B deflection that it too may be superposed upon ascending and descending phases of C and E, and, in the case of intermittent stimulation, there are often superposed the distinct deflections of successive short-lived B-effects upon the lingering descent of the first strong B deflection (slightly exhibited in Fig. 6, Pl. II).

2. Intermittent Exposures to Light. Dark-adapted Eye

For the second set of experiments it was only necessary to interrupt the light before its entrance into the T-tube by a rotary disk of cardboard with eight radial segments cut symmetrically out of it (Fig. 1, e). In order to intercept the light simultaneously from both mirrors situated in the T-tube, the episcotister was set at the point where the light from the Nernst glower, after emerging from a condensor, came to a focus close to the mouth of the T-tube, so that each segment of the rotating disk completely and instantly intercepted the passage of the light in turn. A photographic test in which the beams of light emerging from each end of the cross-arm of the T-tube were reflected side by side onto the photographic paper, exhibited exact coincidence for the make and break of light; so that there was no uncertainty about the eye, which received the one beam, and the photographic register, which received the other, being struck simultaneously at every exposure. It was of course essential for the accurate measurement of the latent periods in the curves that this be so. The records were obtained from dark-adapted eyes exclusively in this set of experiments.

The intermittent phenomena in the eye, corresponding to the rapid interruptions of light by the episcotister, are shown in Figures 5, 6, 7 and 8, Plate II. In Figure 5, a record from animal No. 39, the rate of stimulation was three flashes per second, a frequency slow enough to allow all of the phenomena except the C variations to appear. The first D crest is either absent or masked by the first positive on-effect. The last D deflection is present although so closely superposed upon the last on-effect as to be scarcely perceptible. A slight nick, however, in the ascending slope of the last fluctuation, together with the higher altitude of its summit as compared with those preceding, is evidence of its presence. This fact is an important aid to the interpretation of the curves of higher frequency. The presence of the negative preliminary A may be inferred from the fact that the troughs preceding the on-effects are more acute than those which succeed them.

Figure 6, the fifth exposure of a series from animal No. 37, was made to intermittent light having a frequency of 16 flashes per second. The intervals of dark are slightly longer than the intervals of light, the former being 0.037 seconds and the latter 0.029 seconds on an average. Since there are 32 crests in the curve corresponding to the 32 flashes of light, they may represent either the make or break of light or else an additive effect of both. An analysis of the curve will be found on

p. 388, in section C of division V. When the frequency was increased from 16 to 28 stimulations per second, the eye ceased to respond with individual currents and the general effect produced was the same as that for continuous light, and is exhibited in Figure 7.

In order to determine whether the eye by this time might not be fatigued, the frequency was lowered to 25 flashes per second, and it was found that the responses of the eye, although feeble, were again recordable. The sensitiveness of the galvanometer during this series is shown in Figure 8, a curve produced by a constant current having a strength of one millivolt.

IV. SUMMARY OF OBSERVATIONS

1. The photoelectric phenomena in the eye of the fish (pike) are quite similar to those in the frog. The current of rest flows from cornea to back of eyeball in the outer circuit, a direction designated as positive. The onset of light evokes in the curve a negative preliminary depression A, a positive deflection B and a secondary positive effect C. The withdrawal of light occasions a single positive deflection D.

2. The initial depression A appears only after a certain amount of dark adaptation; its magnitude is relatively small, and it has a latent period of 0.032 seconds.

3. The B deflection is feeble in a light-adapted eye, but with ten to twenty minutes' adaptation to darkness it becomes five to seven times as large. Its latent period is 0.075 seconds and its maximum is reached on an average in 0.26 seconds.

4. The C deflection appears only in a thoroughly dark-adapted eye; its development is slow and becomes ever slower with every exposure to light. Its magnitude may, after long adaptation to dark, exceed that of B by two or three times. In one measured instance the summit was reached in seven seconds.

5. The off-effect D increases in size with the length of previous illumination of the eye. Its latent period is 0.049 seconds and its summit is reached in 0.165 seconds.

6. To intermittent light the eye yields separate responses up to a frequency of 25 flashes per second. At 28 per second the responses become blended together so that the curve obtained resembles that produced by exposure to continuous light, the A and B deflections of the first on-effect and the last off-effect alone being distinguishable.

7. A change in the direction of the current of rest may occur through

changes in the physiological state of the fish forerunning death, or by gradual change in the polarity of the electrodes.

V. DISCUSSION

A. Criticism of the Method

The factors which tended to vary throughout the experiments were the contact of the electrodes with the eye, the tension of the string of the galvanometer, the intensity of the light and possibly the oxygen supply. The temperature of the water conducted into the fish's mouth was that of ordinary tap water and varied from 15 to 17 degrees centigrade. Although in cases of extreme changes the temperature may decidedly alter the phenomena [Gotch (9), Nikiforowsky (18)], this fluctuation from day to day was too slight to modify the reactions in any way. The fish, bound to the board so as not to injure it by too strong compression, was capable of slight muscular movements, and these together with the respiratory action of the gills, affected the contact occasionally between the electrodes and the eye; but as muscular movements of a disturbing character were only occasioned by lack of oxygen, when the water supply was regulated properly the fish lay quiescent throughout the experiment. In order to obtain the best results the tension of the galvanometer string often had to be altered in the course of the same experiment, loosened when maximum throws were desired and tightened when the fleeting currents set up by a high frequency of stimulation demanded a quicker response. Since the light from the Nernst glower was reflected into the eye by means of a small adjustable mirror located inside the dark chamber, the intensity varied to some extent with every readjustment.

The specific effects which an increase in light intensity has upon the curve are summed up in the recent work of Brossa and Kohlrausch (1) in these words:

> Die Latenz wird kürzer, der negative Vorschlag tritt erst von einer bestimmten Lichtintensität auf und wird von da an stärker ausgeprägt. Alle drei positiven Stromschwankungen . . . . steigen rascher und steiler an; die Eintrittsschwankung und die Verdunklungsschwankung erreichen früher ihren Gipfel. Die Senkung zwischen positiver Eintrittsschwankung und sekundärer Erhebung wird tiefer. Die Verdunklungsschwankung steigt in immer schärfer werdendem Knick von der Abszisse aus an. Wohl gemerkt gehen alle diese Formveränderungen Hand in Hand mit einer Zunahme der Ordenatenhöhe der Ausschläge.

Thus an increase of light intensity produces a shortening of the latent periods, a steepening of the slopes of the deflections and an increase in their altitudes.

Although these variable factors probably affected the finer character of the curves to a certain degree as just mentioned, they did not alter their general structure nor the direction of the deflections. In the following discussion which deals with the structural features of both simple and intermittent curves rather than with their magnitude relations, the latter will for the most part be left out of consideration.

B. Comparison With Results Obtained for Other Animals

If we compare the curves obtained from the eye of the pike with those obtained from the frog, we find that for both simple and inter-

TABLE II.

A comparison of the latent periods of the photoelectric reactions for the eyes of the bleakfish, frog and pike.

INVESTIGATOR	ANIMAL	LATENT PERIOD OF A DEFLECTION	LATENT PERIOD OF B DEFLECTION	FROM MAKE OF LIGHT TO MAXIMUM OF B DEFLECTION	LATENT PERIOD OF D	FROM BREAK OF LIGHT TO MAXIMUM OF D	BLENDING RATE FOR INTERMITTENT STIMULATION
v. Brücke and Garten	bleak	0.074″ 0.075	0.128″ 0.132				
Piper	frog	0.045	0.085	0.3″	0.05″	0.18–0.27″	15 per sec.
Day	pike	0.032	0.075	0.26	0.049	0.165	28 per sec.

mittent stimulation the phenomena are closely similar. The corresponding deflections are all like in sign. In Table II are given for comparison the latent periods for the A, B and D deflections, together with the time required for the B and D crests to attain the maximum points in their courses.

The first set of values is that obtained by v. Brücke and Garten (2) on the bleakfish (Bleie), the second set by Piper (20) on the frog, and the last by myself on the pike.

Since the same string galvanometer had been used by Piper as was used in the foregoing experiments, it is noteworthy to compare especially

the last two sets of values. It should be first remarked however that v. Brücke and Garten employed a smaller and considerably less sensitive string galvanometer than Piper did. Furthermore, since they succeeded in obtaining but two photographic records for the bleak, measurements of which are given in the table, their results are to be accepted with reserve.

Turning then to the second and third sets of values, it will be seen that both the latent periods and the times required for the on- and off-effects to reach their maxima, are greater in the frog than in the fish; also that the amphibian eye ceases to respond to intermittent stimuli at a lower frequency than the fish eye does.

These facts might lead one to suppose that the photoelectric processes in the visual organ of the pike were of a more rapid nature than those of the frog. Considering the facts however that the values for the fish were obtained from eyes *in situ* and still in communication with the circulatory system, while those for the frog were obtained from extirpated eyes; and that Piper employed a light of stronger intensity (five ampere arc) than I did, the values in the table are not so closely comparable. The interruption of the circulation in the eye through extirpation presumably occasioned a retardation of the photoelectric reaction, while the higher intensity probably had an accelerating effect which tended to shorten the latent periods; but there is no way of estimating how much the acceleration compensated for the retardation. As a matter of opinion, I believe that the compensation was not enough to make up for the depression in irritability induced by impairing the metabolic activities of the retina.

The value 28 in the last column, the frequency of intermittent flashes at which a blending of the individual deflections in the retinogram occurred, was not an average but was a single value obtained in the course of one experiment upon a normal vigorous animal. Experience taught that much depended upon the tension of the string in the galvanometer in securing the fleeting currents of the eye. Thus currents of high frequency to which the slack fiber would not respond, could be recorded on stretching it tighter.

Piper (20) found that dove and buzzard eyes responded to as high as 40 flashes per second, the chicken to 35, the owl to 20, the rodent to 25, the cat and dog both to 25 and the Makakus Rhesus monkey to 17. That the fish, then, a cold-blooded animal, should possess an eye which reacts with greater rapidity to photic stimuli than do those of warm blooded animals like the rabbit, cat, dog or monkey, seems

incredible, because the fact that the rate of nerve transmission in the cold blooded forms is slower, would lead one to expect a correspondingly slower reaction on the part of the retina as well. Further, the fact that the latent periods for the on- and off-effects in the eyes of the mammals mentioned are about twice as short as those for the fish, would likewise indicate that only minimal intermittent values have as yet been obtained for the warm blooded animals, because theoretically the animal with the shorter latent period is physiologically better capacitated to react to a higher rate of intermittent stimulation than an animal with a slower reaction period. Since various drugs were employed by Piper in his operative methods, such as ether for narcotizing, curare for inhibiting muscular movement, and atropin for enlarging the pupil, these may have played a rôle in diminishing the sensitiveness of the eye to light. As to the use of atropin, Gotch (9) states definitely that it lengthens the latent periods. Other factors, too, such as the tension of the galvanometer string, the resistance of the electrodes, the intensity of the light, the physiological condition of the animal, etc., enter in to make difficult a close comparison between the results obtained by Piper on birds and mammals and those here given for the fish.

C. Synthesis of the oscillatory curve

An analysis of the deflections produced in the retinogram by intermittent stimulation has been carefully made by Piper (20). He regards the curve first from the standpoint of photoelectric currents set up by a series of "flashes" of darkness, and again as compounded of the serial effects from a sequence of flashes of light. By both methods of approach he arrives at the conclusion that the curve consists chiefly of negative preliminaries plus positive off-effects, i.e., of A and D deflections alternating in rapid succession. If the rate of stimulation is slow, the B deflections predominate; but since with increase of frequency the B effects diminish in amplitude while the A and D deflections retain practically their original size, the curve comes gradually to be composed of the last two kinds of deflections. His argument is based upon three facts revealed by measurement on the intermittent curve: first, the time elapsing from each make of light to the beginning of each depression is identical with the length of the latent period for the negative preliminary, A; second, the period elapsing from break of light to the maximum point of the next succeeding depression is identical with the latent period for the positive off-effect, D; third, when an intermittent

series ends in an off-effect, the last deflection, positive, corresponds in length of latent period to that of a deflection appearing at the end of a continuous exposure, thus making the two identical. In the oscillatory curve of the frog, therefore, it is the A and D deflections which primarily determine the oscillations when the frequency of stimulation is relatively high.

From a similar analysis of my own curves I have arrived at a like conclusion in regard to the synthesis of the intermittent curve for the eye of the fish. Figure 6, Plate II, will serve to illustrate. As the duration of each flash in this curve is about the same as that of the last flash in Figure 5, Plate II, which, as has already been pointed out,[3] occasioned a superposition of the D deflection on top of a B deflection, one may infer that such is probably the case here too, every upward stroke being compounded of the ascending slopes of the B and D elevations. There is however another factor involved which tends to diminish this additive effect. It is the negative preliminary A, which exerts a depressing influence upon the positive phases of the curve in the following way: since the sum of the interval of darkness following a stimulation plus the latent period of the next ensuing A deflection (i.e., 0.029 + 0.02″) is always less than the latent period (0.09″) needed by the off-effect to arrive at a maximum, the A depression curtails the full additive value of each off-effect. Therefore the summits of all the upward deflections in the curve, as soon as the effect of the first strong positive on-effect has subsided enough to allow them to appear, are necessarily submaximal up to the last one; but this final one, because of the fact that no A deflection follows to depress it, rises to a maximum. The more the rate of stimulus is increased, the more the A deflections overlap the B deflections and tend to diminish their altitudes.

Further, since the altitudes of B and D deflections both are dependent upon the restorative periods of dark and light preceding each respectively, according as these periods are shortened the altitudes of the crests are diminished. It follows then that two factors, one of interference and the other a diminution in the length of the restorative periods after each stimulation, combine to depress the crests of the waves to a common level as the frequency of stimulation is increased. The rise at the end of an intermittent series is an expression of the release from the depressing effect of a subsequent negative preliminary.

[3] See p. 382.

D. Interpretation of the retinogram

It is generally conceded that the C deflection can play no part in producing the sensation of light, because of the slowness with which it develops. The underlying cause for this deflection might be one of several phenomena which take place in the eye: the chemical dissociation of visual purple, the migration of pigment, the contraction of cones or the chromolytic changes said to occur in the ganglion cells of the retina.

As to the first phenomenon, Kühne and Steiner (16) state that the photoretinal currents occur in the frog after the visual purple has been bleached out. This is confirmed by Holmgren (14). The former authors also claim that if during dark adaptation the re-formation of visual purple be prevented by lowering the temperature, the eyes do not then exhibit any gain in sensitiveness, but react like light-adapted eyes. From the general nature of the statements, however, it is impossible to say whether it is the B and D deflections alone which are affected, or whether C is also.

Since visual purple and the C deflection are both characteristic for dark-adapted eyes of many animals, two questions arise: first, whether visual purple is present in the eyes of all animals whose retinas, when dark-adapted, yield the C deflection upon exposure to light; and second, whether the C deflection is obtainable from all retinas which exhibit visual purple. An answer to these queries must await the accumulation of more experimental data on the subject.

In regard to whether the migration of pigment offers a basis for the explanation of this slow-developing fluctuation in the curve, the number of facts at hand from which to judge is meagre. The migration of pigment and the C deflection are both elicitible from the dark-adapted eye of the fish, frog, dove and lobster. In the retina of the dog on the contrary, neither phenomenon occurs, for, according to Chiarini (4), there is no evidence of photokinetic activity in the pigment, and upon the statement of Piper (20) the C deflection is either entirely absent or else is very small. In the pig's eye the migration of pigment is very slight according to Van Genderen Stort (21); and in the case of the cat the same thing is probably true, judging from the scant amount of pigment present in the epithelial layer. No retinogram has ever been made for the pig, but for the cat v. Brücke and Garten (2) and Piper (20) have photographically recorded pronounced C deflections. Although Hesse (11) among others has described a migration of pig-

ment in the cephalopod eye under the influence of light, the photographic curves of the photoelectric effects obtained by Piper (20) from cephalopod eyes reveal not the slightest trace of a C deflection.

Thus the evidence, although inconclusive because the two phenomena have not been studied side by side in the same animal and under similar conditions, makes it rather improbable that the photokinetic activity of the pigment underlies the phenomenon of the C deflection in the retinogram. A study of the photoelectric effects in the eye of an albino rabbit where pigment is entirely lacking, would throw direct light on the problem. For the common rabbit the C crest has already been recorded photographically by Piper (20).

The contraction of the cones of the retina, observed for the eyes of the fish, frog, salamander, lizard, dove and pig [Van Genderen Stort, (21); Chiarini (3, 4); Hertel (10)] might come into consideration in seeking an explanation for the C deflection, were it not for the fact that, although the latter phenomenon occurs in the arthropod eye [v. Brücke and Garten, (2)], no such contraction of retinular elements takes place in it under the influence of light [Parker (19); Day (5)]. In palaemonetes Parker claims that, although the "proximal pigment cells" undergo no change in length through exposure to light, the "distal pigment cells" become contracted, and the "accessory pigment cells" exhibit migratory activity. But if, as has previously been shown, the mechanical movement of pigment granules can not account for the C deflection, it is no more to be expected that any other kinetic activity, whether of contraction or the shifting of cellular elements, should account for it. It is more probably to be explained upon the basis of a chemical reaction of some substance such as visual purple, or possibly in terms of the chromolysis in the retinal ganglion cells which, according to the observations of Chiarini (3, 4), occurs under the action of light. Until the substance can be definitely identified which underlies the phenomenon of the C deflection, it goes by the general designation of the "Third Substance" in the theories of Einthoven and Jolly (8) and of Piper (20).

In regard to the other deflections in the curve it is impossible from the experimental data at hand to conclude just what their relations to the visual sensations are. Ishihara (15) has argued that they can not be correlated with sensations, first, because they are of too brief duration to represent continuous sensations, and second, because the D deflection which should represent a sensation of darkness is the same in sign as the B deflection which theoretically represents the sensation of light. But the premises upon which these conclusions are based

are debatable issues themselves. The fact that the B and D deflections are alike in sign does not necessarily exclude all correlation between them and the sensations of light and darkness respectively, if one assumes them to be produced by the reactions of two substances which, although reacting with like directional sign as to the electric currents produced, possess a qualitative difference which the galvanometer is incapable of expressing. Were the instrument, for example, to record the B deflection as a red curve and the D deflection as a blue one, the directional sign remaining the same, any such qualitative difference would immediately be apparent; thus red could be associated with the sensation of light and blue with that of darkness.

As to the first argument, the premise that sensations of light are continuous, is invalid in the sense of an unvarying continuity. It was however evidently with that sense in mind that the argument was formulated. According to Ishihara, for example, the sensation which a person experiences when an electric light is turned on is a continued, unchanging sensation of brightness which lasts until the light is turned off. An analysis of such a sensation however discloses the error of the premise. When one gazes at the sky one may think that with respect to brightness the sensation thereof suffers no apparent change; but one needs only to perform two simple experiments in order to convince himself to the contrary.

In a room having a window which commands a free expanse of sky, cover one eye, hold a card in front of the other so as to screen off half the patch of sky, gaze fixedly at a speck on the pane for a moment and then withdraw the card. The half of the sky at which you were gazing is seen to be distinctly dimmer than the one just uncovered. Again, in a very dimly illuminated room, when one gazes at a white spot on a black background, the spot soon vanishes like an extinguished light. The element of fatigue, therefore, both for the retina as a whole and for the fovea centralis, makes quite impossible a continuous sensation of light in the sense of an unchanging sensation. Under ordinary conditions the eye is ceaselessly shifting on its axis so as to bring now this now that portion of an object to fall upon the fovea for close inspection, so that the factor of fatigue is reduced to a minimum.

The sensation of darkness is likewise assumed by Ishihara to be continuous, and that on that account the D deflection can not be the correlative of the sensation. But one may gaze at a black field as well as at the sky, cutting off half of the view with a card, and find upon removing the card that the newly exposed half is comparatively the

darker, thus showing that the sensation of blackness steadily diminishes in intensity as its duration increases.

The decline of a deflection after reaching its maximum altitude might therefore be the expression of the diminution in strength of a sensation, whether of light or of darkness. The ascending phase of a deflection would analogously indicate the growth of a sensation to a maximum. In the foregoing I have had B in mind rather than A as being the representative of the sensation of light; but that the A deflection can not be ignored will be apparent from an analysis of the intermittent curve.

When one compares the threshold value for the fusion of visual images with that for the blending of the oscillations in the intermittent curve, it is found that there is a certain approximation between the two. In the former case the threshold value for the human eye ranges, according to Piper, from 20–50 stimulations per second, at which frequency the intermittent flashes give the sensation of continuous light, while in the latter case the value ranges, depending on the animal, from 17–40 flashes per second, at which the individual oscillations blend into a smooth curve. From these comparative values one may infer that some general correlation exists between the photoelectric phenomena and the visual sensations of light and darkness. They do not however throw much light on the question of the significance of the individual deflections.

If the oscillatory curve is formed synthetically as has been described, then the sensations in the brain corresponding to the oscillations must be due, those of light to the depressions and those of darkness to the crests in the curve; because, from the analysis of the curve, the A deflection plays the chief rôle in creating the depressions and the D deflections the crests. The B deflection here seems to participate but little and consequently has little to do with the sensation of light. Nikiforowsky (18) has shown furthermore that the B deflection vanishes completely when the temperature is lowered to zero degrees centigrade, while the A and D deflections remain almost unaffected. According to this again, the A and D deflections, being the most stable, would represent the sensations of light and darkness respectively; because, were the sensation of light to be attributed to the B deflection, when the latter vanished upon lowering the temperature it would be a case of *reductio ad absurdum*, of an eye capable of rendering a sensation of darkness but incapable of rendering one of light. In the simple curve for the normal eye, however, the B deflection is such a prominent feature

that further investigation is needed to decide whether it can be left entirely out of consideration or not.

The preceding experiments dealt, it must be remembered, with dark-adapted animals (with but one exception), so that both simple and oscillatory curves must yet be studied for the effects of light-adaptation before a definite theory can be well grounded.

E. Similarity of the tri-substance theories

In looking over the literature there have come to my attention certain facts in regard to the theories proposed by Einthoven and Jolly on the one hand and by Piper on the other which are worth drawing attention to. The two theories are supposed to be antagonistic to each other, but they have in fact more in common than has been suspected.

When Piper evolved his theory it was with the object (p. 120) of obviating the necessity of ascribing each fluctuation in the curve to a separate process in the retina, as he suppposed had been done by Einthoven and Jolly. I think however that a careful analysis of the two theories will show that Piper partly misinterpreted the application of the theory of the other two men, and that what he sought to obviate was something which he had imputed to their theory which did not in reality belong there.

Table III will enable us easily to compare the two substance theories. On the left side are given the characteristics of the substances as conceived by Einthoven and Jolly, and on the right as conceived by Piper. Substances II, I and III of Piper's are comparable to I, II and III of the Einthoven-Jolly theory. In each cross comparison it will be noted that the direction of the reaction is the same for the lighting effects and also for the darkening effects and further that the latencies of reaction for the several substances likewise correspond in the matter of relative duration. During illumination the curves for Piper's substances II and I, after having attained a maximum positive and negative level respectively, remain constant on that level up to the off-effect, whereas, according to Einthoven and Jolly they do not remain constant, substance I continuing in the original direction and becoming more and more negative, and substance II changing direction after reaching a maximum positive value and returning to zero. Except for this difference in the continuous effects during the exposure, the three substances of the one theory have identically the same characteristics as

the three corresponding ones of the other theory. Thus Piper's substance II is nothing more nor less than substance I of Einthoven and Jolly, and vice versa. The third substance is identical for both.

An apparently radical difference between the two theories comes to light when the theoretical reactions of the substances are applied

TABLE III.

The tri-substance theory of Piper compared with that of Einthoven and Jolly.

EINTHOVEN AND JOLLY		PIPER
Substance I		Substance II
Lighting effect	negative	negative.
During exposure	increasingly negative	constant negative level.
Darkening effect	positive	positive.
Latency of reactions	relatively short	relatively short.
Requirements	light eye exposed to a flash of darkness	
Substance II		Substance I
Lighting effect	positive to a maximum	positive to a maximum
During exposure	diminishingly positive	constant positive level.
Darkening effect	negative	negative.
Latency of reactions	medium	medium.
Requirements	dark eye and short exposure to weak light.	
Substance III		Substance III
Lighting effect	positive to a maximum	positive to a maximum.
During exposure	diminishingly positive	diminishingly positive.
Darkening effect	none	none.
Latency of reaction	long	long.
Requirements	dark eye and relatively strong illumination.	

to the retinogram. In the theory of Einthoven and Jolly the reactions of the three substances apparently occur in sequence, while by the Piper hypothesis they progress simultaneously. It would seem, then, to be a difference between driving horses tandem and driving them abreast. It is true there is a commencement sequence for the reactions of the three Piper substances, but the intervals between the beginning-times

of each are relatively short. In the construction which Piper placed upon the statements of Einthoven and Jolly concerning their curves on the contrary, the reactions of substance I proceeds to its finish before the reaction of substance II begins. This seems at least to have been the conception of the E and J. theory held by Piper when, in referring to it (p. 120), he judged it as physiologically impossible because it demanded a separate retinal process to account for each variation in the curve. In opposition to this idea it appeared therefore more probable to Piper that the fluctuations in the curve were interference phenomena resulting principally from two almost simultaneous reactions acting in opposite directions and at slightly different rates; and upon the basis of this conception he evolved a graphic schema by which he explains the genesis of both simple and oscillatory curves.

Although this interference conception was developed and formulated into definite terms by Piper, its essence was nevertheless present in the theory of Einthoven and Jolly. That these investigators had also conceived of interference occurring between overlapping reactions will be seen from the following quotations (in which all italics are mine).

"The lighting effect (p. 400) of the first substance . . . does not yet appear in fig. 14, which need not surprise us, since the darkening reaction must appear sooner than the lighting reaction. Nevertheless the lighting effect makes itself appreciable to some extent during the record of the curve; for . . . . it is evident that the summit height in fig. 14 is only little greater than that in fig. 13. The increase is only 6 on 110 microvolts, while the duration of lighting is increased from 1.9 to 3 sec. It is the lighting effect of the first substance which here *hinders* the development of a higher summit B." Again on p. 401, "the summit A, is *superposed* in such a way upon the slow wave C that the form of the waves may easily be recognized separately." On p. 402 the interference conception is more expressly formulated in the statement, "If the duration of the lighting is a little longer and the effects of the other two substances begin to become perceptible, the off effect is determined by the *resultant of three forces*."

From these citations it is clear that the nucleus of an interference hypothesis existed in the minds of Einthoven and Jolly but that it was never developed to the extent that it was later by Piper.

In many of my records the continuous effect of illumination is not expressed in the curve as an undeviating course on a constant level, but by a steady and continuous descent, a phenomenon not interpretable upon the basis of the Piper theory that the reactions of substance I

and II remain in a state of equilibrium during the period of illumination. Either substance I must diminish in the strength of its positive reaction or else the reaction of substance II must steadily grow in strength negatively, in order to account for this fall in the curve which seems to be a constant feature in retinograms of dark-adapted fish eyes. Nor can the difference between the reactions of dark and light eyes, a phenomenon not taken into consideration by Piper in testing his theory, be otherwise accounted for than by the foregoing assumption. I am therefore inclined to agree with Einthoven and Jolly that the reactions of substances I and II, instead of reaching a level of stability, vary constantly, either waxing stronger or growing weaker, throughout the period of illumination.

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DESCRIPTION OF PLATES

The figures in the two plates are tracings from photographic records.

PLATE I

Fig. 1. Experiment 40. Date, '13/III/7. 12.05 p.m. Exposure No. 2. The fish was brought fresh from the market and photographic records were made immediately. Exposure No. 1 yielded a curve similar to No. 2 but the latter was selected for reproduction because of its clearer image. This record was made after the eye had been adapted to the Nernst light for two minutes to counteract the two minute interval of dark adaptation necessitated by the development of exposure No. 1. The off-effect for the light-adapted eye is here seen to be stronger than the on-effect. B and D deflections manifest.

Fig. 2. Experiment 40. Date, '13/III/7. 12.18 p.m. Exposure No. 3, following exposure No. 2 (fig. 1) after 10 min. dark adaptation. The on-effect is now stronger than the off-effect. B and D deflections present and a faint suggestion of A.

Fig. 3. Experiment 40. Date, '13/III/7. 12.27 p.m. Exposure No. 4, following expos. No. 3 after 7 min. more dark adaptation. Deflection A is now fully present, B has increased in strength while D has grown feebler.

Fig. 4. Experiment 39. Date, '13/III/6. 11.33 a.m. Exposure No. 1. Fish dark-adapted over night. A, B, C and D deflections present. Although the D deflection in this exposure is stronger than the B, in the other exposures of this experiment the reverse relation is found. The larger D deflection may, in this case, have been due to a slight condition of light adaptation at the beginning. This light adaptation was not however strong enough to repress the C deflection.

PLATE II

Fig. 5. Experiment 39. Date, '13/III/6. 12.04 p.m. Exposure No. 4. Fish was kept dark-adapted after each of the previous exposures. The illumination, recorded by the dashes in the lowest line of the figure, was intermittent, the rate of stimulation being about three flashes per sec. The duration of a single flash is about 1/6 sec. A, B and D deflections are present. The last D deflection is superposed upon the last B deflection owing to the curtailment of the final period of illumination.

Fig. 6. Experiment 37. Date, '13/III/4. 4.27 p.m. Exposure No. 5. Fish was dark-adapted over night. Rate of stimulation, 16 flashes per second. Intervals of light = 0.029 sec., of darkness = 0.037 sec. To the 32 flashes of light there correspond 32 crests in the curve.

Fig. 7. Experiment 37. Date, '13/III/4. 4.47 p.m. Exposure No. 6. Rate of stimulation, 28 flashes per sec. The individual oscillations have fused and the effect is the same as for continuous illumination.

Fig. 8. Experiment 37. Date '13/III/4. 5.25 p.m. The three deflections were produced by substituting a galvanic battery for the eye of the fish and sending three consecutive shocks through the galvanometer having a current strength of 0.1 millivolt. The current from the eye throughout Experiment 37 gave throws of scarcely half this altitude.

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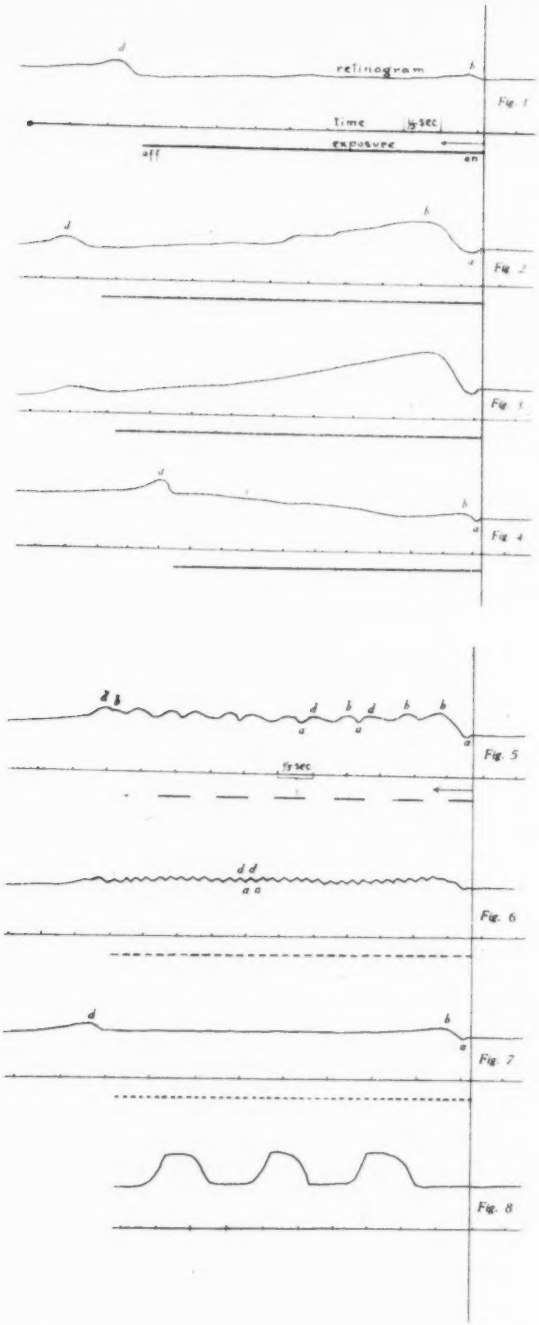

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THE COMPARATIVE RATE AT WHICH FLUORESCENT AND NON-FLUORESCENT BACTERIA ARE KILLED BY EXPOSURE TO ULTRA-VIOLET

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The source of ultra-violet used was a Cooper-Hewitt quartz mercury-vapor burner operating at 170 volts, 3.3 amperes and 2400 candle-power. The non-fluorescent bacteria used were B Colil communis, B. violaceous, B. Proteus vulgaris, B. sulbtis, Sarcina aurantiaca, Micrococcus capsulatus, and B. mucosus capsulatus. The fluorescent bacteria were furnished by the State Water Survey of Illinois. The Water Survey obtained the bacteria from the samples of water sent in from different parts of the state for examination. Mr. Tanner of the Water Survey had not gone far enough in the classification of these bacteria at the time we obtained them from him to furnish the names for them and for that reason they were referred to in this paper as fluorescent bacteria numbers 1, 2, 3, 4, 5, 6 and 7. He had determined, however, that they were all different types of fluorescent bacteria and were non-spore formers.

The liquids containing the bacteria to be exposed to ultra-violet were prepared in the following way. A standard loop of bacteria was taken from a five day old culture and introduced into 25 cc. of 0.8 per cent sterile sodium chloride solution. This liquid was filtered through a fine grained sterile filter. Two cc. of the clear filtrate containing the bacteria were introduced into each of eleven quartz tubes. These tubes were placed 30 cm. from a Cooper-Hewitt quartz mercury-vapor burner operating at 2400 candle power. At intervals of 20 seconds a tube was removed. In this manner tubes containing the bacteria were obtained which had been exposed to the light for 0, 20, 40, 80, 100, 120, 140, 160, 180, 200 seconds respectively. The contents of these tubes were plated with agar and incubated at 37° C. for 48 hours. At the end of this time counts were made according to the ordinary bacteriological method.

Results of these counts may be seen in Table 1 for eight kinds of fluorescent bacteria and in Table 2 for seven kinds of non-fluorescent bacteria. The numbers of colonies for the 20 and 40 second intervals of exposure are not given, because as a rule they were too numerous to be counted accurately. The counts for the numbers of colonies after 0 time of exposure were made possible by diluting the liquid a million times before plating.

It may be seen in Table 1 that there were alive 31 colonies of fluorescent bacteria No. 1, 15 of No. 2, 3 of No. 3, 3 of No. 4, 11 of No. 5, 2

Table I. Fluorescent Bacteria

Time of exposure	Number of bacteria No.1	Number of bacteria No.2	Number of bacteria No.3	Number of bacteria No.4	Number of bacteria No.5	Number of bacteria No.6	Number of bacteria No.7	Number of bacillus pyocyaneus
0 Sec.	72,000,000	80,000,000	28,000,000	70,000,000	75,000,000	33,000,000	25,000,000	79,000,000
10 "	----------	----------	----------	----------	----------	----------	----------	----------
40 "	----------	----------	----------	----------	----------	----------	----------	----------
60 "	750	813	55	210	460	12	361	88
80 "	459	663	29	79	211	10	111	20
100 "	223	221	25	56	115	7	18	14
120 "	154	102	20	29	103	5	8	12
140 "	97	88	15	18	56	4	6	8
160 "	61	77	12	12	30	3	6	2
180 "	43	29	10	6	18	3	5	1
200 "	31	15	3	3	11	2	4	1

Table II. Non-fluorescent Bacteria

Time of exposure	Number of B.Subtilis	Number of B.Mucosus capsulatus	Number of B.Proteus vulgaris	Number of B.Violaceus	Number of Micrococcus capsulatus	Number of B.Coli communis	Number of Sarcina aurantiaca
0 Sec.	18,000,000	24,000,000	295,000,000	27,000,000	28,000,000	28,000,000	195,000,000
20 "	----------	----------	----------	----------	----------	----------	----------
40 "	----------	----------	----------	----------	----------	----------	----------
60 "	13	460	2,480	83	171	378	4,820
80 "	7	112	79	60	4	9	60
100 "	7	107	71	20	2	1	10
120 "	6	8	4	5	2	0	2
140 "	4	6	2	4	1	0	1
160 "	2	5	2	0	0	0	1
180 "	1	0	1	0	0	0	0
200 "	0	0	0	0	0	0	0

of No. 6, 4 of No. 7, and 1 of B. pyocyaneus after an exposure of 200 seconds to ultra-violet while in Table 2 it may be seen that all of the non-fluorescent bacteria were killed when exposed for a similar length of time. This is taken to mean that the fluorescent bacteria are more resistant to the effect of ultra-violet than non-fluorescent bacteria.

The results of these experiments are also expressed in the form of curves in figures 1, 2, 3, 4, 5, 6, and 7. The unit of the ordinate scale is 1 cm. to one hundred bacteria, that of the abscissa is 1 cm. to 20

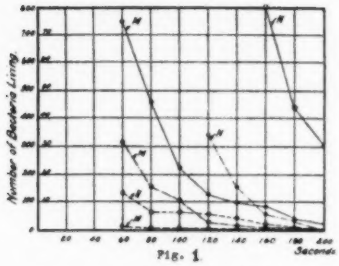

Fig. 1

——— Fluorescent bacteria No. 1
– – – – Non-fluorescent B.subtilis (two days old)
– – – – Non-fluorescent B.subtilis (two weeks old)

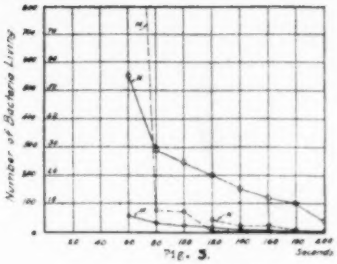

Fig. 3.

——— Fluorescent bacteria No. 3
– – – – B.proteus vulgaris
– – –

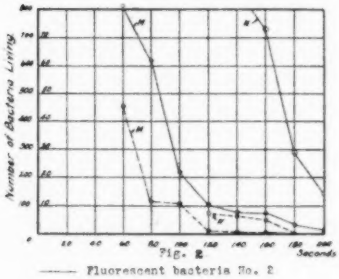

Fig. 2

——— Fluorescent bacteria No. 2
– – – – B.mucosus capsulatus
– – –

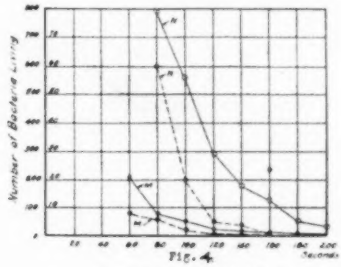

Fig. 4.

——— Fluorescent bacteria No. 4
– – – – B.violaceus
– – –

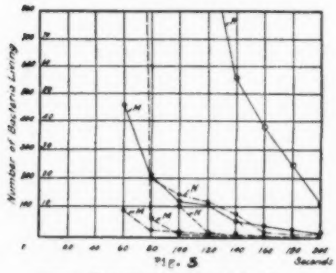

Fig. 5

——— Fluorescent bacteria No. 5
– – – – Sarcina aurantiaca
– – – B.pyocyaneus

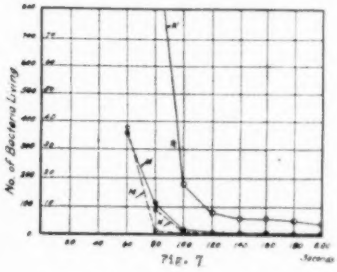

Fig. 7

——— Fluorescent bacteria No. 7
– – – – B.coli communis
– – –

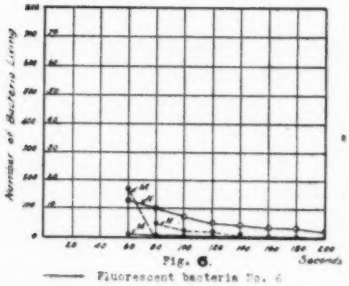

Fig. 6.

——— Fluorescent bacteria No. 6
– – – – Micrococcus capsulatus
– – –

seconds. Each curve marked N is plotted to a scale whose ordinate is 10 times as great as that marked M.

It may be seen from the curves that the non-fluorescent bacteria are killed more rapidly than the fluorescent bacteria. Of the non-fluorescent bacteria B coli was the least resistant to the action of ultra-violet, being killed after an exposure of 100 seconds, while B. subtilis was the mst resistant, being killed after 180 seconds. No attempt was made to determine how long an exposure was necessary to kill all the bacteria in any of the cultures of the fluorescent bacteria.

The questions of most interest to us were why are bacteria killed by ultra violet and why are the fluorescent bacteria more resistant than the non-fluorescent. Dreyer, Hanssen[1] and others found that the ordinary proteins such as serum albumin, serum globulin, egg albumin and egg globulin, etc., are comparatively easy to coagulate by ultra-violet. In view of this observation it seemed to us that the most plausible explanation for the bactericidal action of ultra-violet is that the short waves coagulate the protein or protoplasm of the bacteria and by this means kill them. This assumption while explaining the bactericdical action of the short waves does not, however, explain the fact that fluorescent bacteria are more resistant to the action of ultra-violet than the non-fluorescent.

Von Graefe and Helmholtz[2] showed that the lens possesses the property, fluorescence, by which it converts the absorbed short waves into longer waves and gives them off in this form. Burge,[3] while carrying out experiments in an attempt to determine the part played by ultra-violet in the production of glass blower's cataract, found that the lens protein differs from most of the other proteins in that it is very difficult to coagulate by means of ultra-violet. He also showed that the lens protein could be modified by the salts of calcium and magnesium and by sodium silicate, substances found to be greatly increased in human cataractous lenses, so that the short wave-lengths of the spectrum could coagulate it and hence produce an opacity of the lens or cataract. He found that these salts decrease the fluorescence of the lens at the same time that they render the lens protein easily coagu-

[1] Comptes Rendus, 1907, cxiv, 234.

[2] Von Graefe and Helmholtz: Ueber die Fluoreszenz der retina. Poggendorff Annalen der Physik und Chemie, Bd. xciv.

[3] Burge: This Journal, 1914, xxxvi.
Hanssen: Comptes Rendus, 1907, cxiv, 234.

lable by ultra-violet. This last observation suggested that there might be some relation between the fluorescence of the lens and its great resistance to the coagulative effect of ultra-violet. To explain the great resistance of the lens protein the assumption was made that the lens by converting the short waves into longer waves and giving them off as such disposes of the energy of the absorbed short waves, which otherwise would have been spent in coagulating its protein. The fact that these substances which render the lens protein more easily coagulable by ultra-violet at the same time decrease the fluorescence of the lens would seem to lend support to this hypothesis.

To explain the great resistance of fluorescent bacteria to the action of ultra-violet an assumption is made similar to that made in explaining the resistance of the lens protein to the action of ultra-violet, namely that fluorescent bacteria protect themselves from the coagulative effect of ultra-violet by converting the short wave-lengths into longer waves and hence dispose of the energy of the absorbed short waves. The non-fluorescent bacteria, however, are unable to dispose of the energy of the absorbed short waves and for this reason they are killed more easily by ultra-violet than the fluorescent bacteria.

THE EFFECTS OF CHANGE IN AURICULAR TONE AND AMPLITUDE OF AURICULAR SYSTOLE ON VENTRICULAR OUTPUT

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What we know concerning the role of the auricles in the dynamics of the heart has been largely a matter of inference: the inference being that the auricles have a passive and an active role; acting passively as reservoirs for the accumulation of blood which is to pass into the ventricles, and actively as pumps, injecting blood into the ventricles and producing a better ventricular filling. The importance of good ventricular filling is apparent when we consider that the ventricle, within certain limits, tends to expel with each systole most of the blood it receives in the preceding diastole. The auricles regulate indirectly by their contraction, the volume output of the ventricles.

To what extent blood pressure and ventricular output are affected by auricular systole has not been a subject of much experimentation. Henderson (1) using the cardioplethysmograph on the heart of the dog, came to the conclusion that auricular systole had practically no filling effect on the ventricles.

With the use of another method, I showed (2) that auricular systole occurring at the normal time interval in the cardiac cycle, increased the blood pressure by 55 per cent of that maintained by the filling action of venous pressure alone. While the filling effect of auricular systole on the ventricles was not directly recorded, it seemed a logical inference, from the experiments then performed, that the increased ventricular output was due largely to an increased ventricular filling. However, at the time, it was considered probable that auricular systole affected ventricular output in other ways. Also it was argued

How many factors are there at work in the interval of auricular systole under consideration causing these large blood pressure changes. Whether the relative amounts of blood forced into the ventricles, and the varied perfection of valvular action alone can account for the changes, is very difficult to say.

The physiological condition of the ventricular muscle at the moment of ventricular stimulation may be of considerable importance. It is a well known fact that a properly stretched muscle gives a far more efficient contraction to a given stimulus than a relaxed muscle. The same probably holds true with ventricular muscle, without in any way being at variance with the 'all or none law.' Straub's records clearly show that the tension of the ventricles is markedly increased by auricular systole, and lasts a short but appreciable time. This period of tension may be of considerable importance. Probably that auricular systole producing it just at the moment the ventricular impulse reaches the ventricles is the most efficient systole in producing the largest end result, namely, ventricular output.

It was to determine the relative importance of increased ventricular filling, perfection of valvular action, and presystolic intraventricular pressure, on ventricular output, and also to determine if other factors are at work, that this research and others concerning the role of auricular systole were undertaken. The experiments reported in this paper have primarily to do with ventricular filling and valvular action.

For purposes of comparison, the method used in the previous work will be briefly reviewed.

The heart of the dog was exposed and independence of the auricles and ventricles established by crushing the auricular ventricular bundle with the Erlanger heart clamp. Arterial and venous pressures, and contractions of the auricles and ventricles were recorded. The ventricles were stimulated at approximately the rate of auricular contraction. By such stimulation "interference waves" similar to interference waves of sound, were produced. That is, the auricles and ventricles at times would beat in accord and at other times in varying degrees of interference. Auricular systole was placed in different phases of ventricular cycle; at one time occuring during ventricular systole, another time early in ventricular diastole, and at another time immediately before ventricular systole.

The method showed that the effects exerted by auricular systole on increasing ventricular output vary with the place occupied by auricular systole in ventricular cycle. The auricular systole placed at about the normal time interval in the normal heart cycle was found to be most effective. In these experiments the magnitude of auricular systole remained constant, the varying factor was time—the time interval at which auricular systole occurred in ventricular cycle.

In the present method, for a particular reason the heart of the river terrapin was used. The auricles of that heart show two distinct types of contraction, the rapid clonic and the slow tonic. The clonic are superimposed upon the tonic, and, as a rule, vary in amplitude, inversely

as the height of the tonic contraction. These properties of the auricles allow the studying of the effect of auricular systole on cardiac dynamics in an entirely different way from that of the previous method mentioned. It permits a study in which the magnitude of auricular systole is the varying factor, while the time interval at which auricular systole occurs in ventricular cycle is the constant factor—the direct opposite of conditions prevailing in the experiments in Method I. In Method I, the possibility of variation in the perfection of valvular action, until eliminated, might be considered a possible controlling factor. In Method II, where auricular systole precedes ventricular systole by the same constant time interval, this possibility is reduced to a minimum.

With changes in auricular tone, the auricular output varies greatly. Therefore, it was thought that if ventricular output varied as a result of auricular tone changes, these variations could be ascribed primarily to different filling effects of the auricles on the ventricles, rather than to variations in valvular action.

To determine whether ventricular output varied with auricular tone, the method represented in the schema was employed.

The heart was perfused in situ with Ringer's solution. The contractions of both auricles were recorded and the ventricular output measured directly. The left Cuvierien duct and hepatic veins were ligated and a cannula of maximum capacity was inserted in the right Cuvierien duct. This cannula was connected with a Marriotte bottle which delivered the Ringer's solution to the base of the heart at any constant pressure. All the arteries coming from the truncus arteriosus with the exception of one were ligated. This artery was connected by cannula with a differential volume recorder.

This recorder consists of a three-way connecting tube with two long vertical arms running upward and one short one running downward. The short vertical tube is supplied with a stop cock. Into one of the long vertical tubes the volume flow to be measured is led. In the other tube is a cork float carrying a glass writing point which records the height of the liquid in the recorder. The stopcock is so regulated that the mean volume flow when led into the recorder will maintain the level of the liquid at about the center of the tube. When the volume flow decreases, the outflow is greater than the inflow, the level of the liquid falls to a point where its pressure will reduce the recorder outflow to the recorder inflow and maintain the writing point at a lower level. When the volume flow increases, the reverse

occurs: the liquid rises until by its increased height it produces a recorder outflow equal to the recorder inflow. The sharpness of the curves indicate the suddenness of change in volume flow.

Without exception all the hearts experimented upon showed marked oscillations of auricular tone. The frequency of the waves varied with the animal. In some experiments in which oscillations were infrequent additional ones were artificially produced either by mechanical irritation (tug on the auricular suspension), or by flooding with a few ccm. of cold Ringer's solution. The results in all cases were the same.

Numerous records were made with venous pressures varying from 10–60 mm. of water. Tone waves occurring with all these pressures had an effect on ventricular output. When the higher venous pressures were employed, however, the tone waves were not nearly as marked, and changes in ventricular output accompanying auricular tonus changes were correspondingly less marked. With venous pressure at about 25 mm. of water the tone waves were most marked and consequently with this pressure the effects of varying amplitude of auricular systole were best studied. This pressure, apparently, permitted heart action as normally seen in the exposed heart of the turtle.

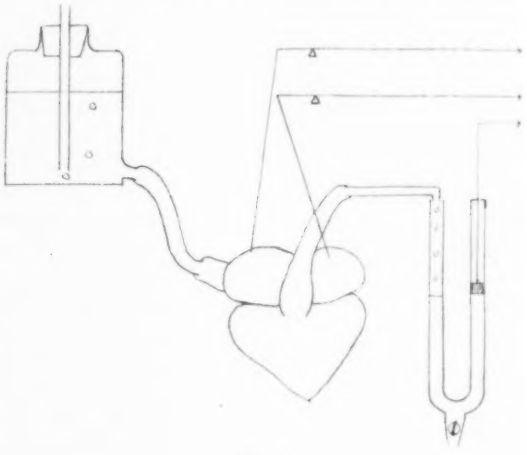

Fig. 1.

Figures 2 and 3 show the usual effects of changes in auricular tone on ventricular output. The corresponding points of the curves are marked. In the case of figure 1 the decreased ventricular output occurring with increase of tone is large. Before the onset of a tone wave, the output was 31 ccm. per minute. This decreased to 10 ccm. per minute. The decrease in ventricular output occurs immediately with the onset of the tonic contraction of the auricles, but becomes more

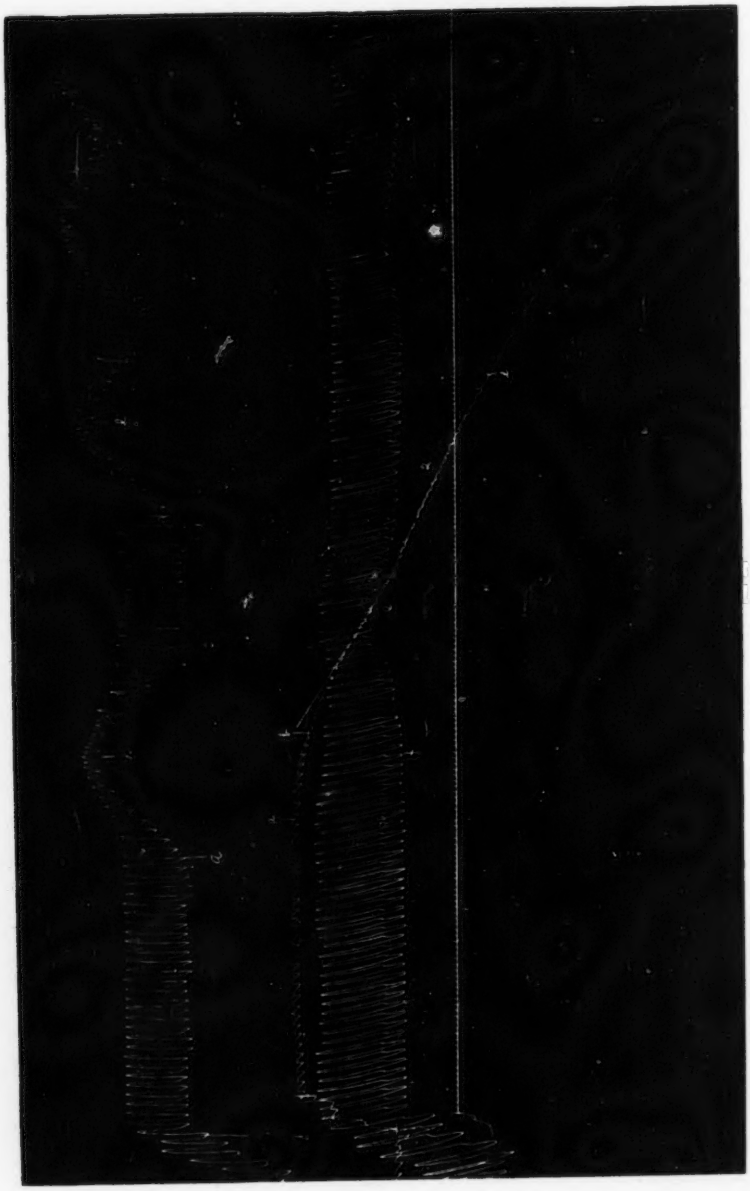

Fig. 2.

Fig. 3.

marked at the crest of the tonic contraction. The late appearance of the marked decrease undoubtedly is due to the masking effect of the tonic contraction itself. The tonic contraction is fairly rapid, and the filling effect resulting from it astonishingly effective. In some cases where the tonic contractions are exceptionally large, the volume output of the ventricles may be increased during the period of shortening of the tonic contraction, even though the amplitude of the individual auricular systoles are progressively decreasing: the decreased amplitude of auricular systole is largely compensated by the effectiveness of the tonic contraction. The marked decrease of ventricular output occurs where the tonic contraction has reached its crest and the ventricles are filled only by venous pressure and small auricular systoles.

Figure 3 shows the decreased volume output followed by partial recovery. The early compensatory effect of the tonic contraction for the decreasing amplitude of auricular systole is shown again. After the completion of the first part of the tonic contraction, which is the most effective, the volume output decreases rapidly and begins to increase again only with the relaxation of the tonic contraction and increase in amplitude of auricular systole. The increase in volume flow is very gradual, corresponding with the gradual increase in the amplitude of auricular systole. The volume flow never reaches the initial volume output for two reasons: the initial auricular amplitude does not return and the auricle remains in higher tone, consequently having a smaller capacity, which in turn reduces the auricular output. The objection might be raised that the reason for the rapid decrease in ventricular output occurring during the relaxation phase of the tonic contraction is to be found in a diminished venous pressure at the base of the ventricle caused by blood accumulating in the easily dilated auricles. With this objection in mind, a cannula which could deliver from 10-30 times the ventricular output was inserted into the right Cuvierien duct. The very gradual return of volume output occurring with the gradual return of initial auricular amplitude in figure 2 speaks against this objection.

In order to be in a position to ascribe changes in ventricular output to accompanying changes in auricular tone as due purely to an auricular effect, it is necessary to demonstrate that the ventricle itself is not participating in the tone changes. Measurements of the length of ventricular fiber with a myocardiograph were not made, because of the danger of interfering with ventricular contraction and because rhythmical variation in length of ventricular fiber occurring synchron-

ously with oscillations of auricular tone would not necessarily be an index to *true* tonus changes, but rather to differences in the filling of the ventricle occurring during various stages of auricular tone oscillation.

From the experiments I performed, it seemed reasonably safe to assume that true ventricular tone changes did not accompany or follow tone changes in the auricles. The number of tone oscillations in the right auricle were much greater than those in the left. The right and left auricles though much more intimately connected with each other than the auricles and ventricles, seldom showed synchronous tone oscillations. When the oscillations were synchronous, one could not feel sure whether the wave was conducted from one auricle to the other or whether it occurred independently in both auricles.

Rosenzweig (3) states that tonus waves *may* occur synchronously in both auricles and in auricles and ventricles. Fano (4), however, in 100 experiments found the ventricle to show tone oscillation in only three cases.

In view of these facts, and of the fact that ventricular output invariably is affected by changes in auricle tone, it would seem fair to ascribe these changes in ventricular output to auricular tone changes.

Henderson and Johnson (5) in their discussion of valve closure by back pressure and broken jet, state that my previous results may be adequately explained by variation in perfection of valvular action, the valve at times closing by hinge movement from back pressure with considerable regurgitation of blood. This explanation of my results loses much of its force in the present experiments where auricular systole precedes every ventricular systole by the same time interval, thereby exerting its broken jet action on valve closure at all times at the proper moment.

The auricular effects in these experiments apparently are not due to variations of valvular action but to:

1. Variation of auricular diastolic volume. This is a factor of prime importance since it permits a varying auricular output, provided the auricles tend to empty themselves with every contraction and do not meet with materially increased resistance when the auricular output is large than when it is small.

2. Variation in the ability to develop tension in varying phases of tonus oscillations. Patterson, Piper and Starling (6) place considerable importance on the relation between the initial length of mammalian ventricular fiber and tension developed in ventricular systole. If the auricle of the turtle shows the same relation, the auricular sys-

tole occurring when the auricular tone is low should be able to overcome more tension and fill the ventricle better. (I am at present investigating this question.)

1 and 2 would work in the same direction by producing a better filling of the ventricle and in turn an increased efficiency of ventricular fiber.

If presystolic intraventricular tension is of any importance, this factor would vary inversely with the height of tone and work in the same direction as the other auricular effects.

SUMMARY

The effects of variation of amplitude of auricular systole on ventricular output were studied on the heart of the river terrapin.

Because of the oscillations of auricular tone, this heart is peculiarly adapted to this research.

Perfusion of the heart with a constant venous pressure showed variations in ventricular output resulting from oscillations of auricular tone.

Reasons are given for considering variation of valvular action as a minor or negligible factor.

The variations in ventricular output are attributed mainly to the direct effects of variations in ventricular filling.

The slow tonic contractions as well as the rapid clonic contractions of the auricles exert a filling effect on the ventricle in proportion to the amplitude of these contractions.

The decreasing effect of auricular systole of decreasing amplitude occurring during the shortening phase of the tonic contraction is masked by the filling effect of the tonic contraction.

The increased ventricular output resulting from auricular systole of large amplitude is discussed and attributed to

a. Large auricular content at the disposal of auricular systole, this tending to increase the auricular output or ventricular filling.

b. Greater length of auricular fiber, this increasing the efficiency of the auricular contraction and therefore the volume of auricular output, and to

c. Greater ability to increase the length and tension of the ventricular fiber, thereby producing a greater efficiency of ventricular systole.

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